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BY

R. W. MARSH

AND

I. THOMAS

WITH THE ASSISTANCE OF

F. C. BAWDEN H. MARTIN
D. J. FINNEY D. J. WATSON

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ERRATUM

Annals of Applied Biology, **36**, 4.

Page 551, last line but one: *setting* should read *wetting*.

THE COMPETITION BETWEEN BARLEY AND CERTAIN WEEDS UNDER CONTROLLED CONDITIONS

IV. COMPETITION WITH *STELLARIA MEDIA*

BY HAROLD H. MANN AND T. W. BARNES

Woburn Experimental Station, Husborne Crawley, Bletchley

Chickweed (*Stellaria media*) is one of the commonest annual weeds on almost all soils, and forms 20–30% of the weed herbage on the lower greensand soils of Woburn, Beds. It occurs in all crops in this area except on very acid soils.

When barley and chickweed are planted together, with abundance of water and nutrients for both, it is found that increasing the density of planting of the barley reduces the loss due to chickweed competition, but even with very close-planted barley, the loss caused by the weed amounts to nearly two-thirds of the total fresh weight and to four-fifths of the grain yields. This is much greater than with the two annual weeds previously studied (spurrey and mayweed). In a sparse crop of barley, increase in chickweed growth may reduce the barley to less than 10% of its growth without the weed, while the chickweed itself is relatively little affected by the presence of the barley.

There is no evidence of any specific effect of the roots of the one plant on the other; they intertwine without any sign of attraction or repulsion between them.

Comparisons are made with other annual weeds previously studied, and it is suggested that the method adopted furnishes a means of assessing the relative effectiveness of these weeds in competing with barley.

INTRODUCTION

In a previous paper in the present series (Mann & Barnes, 1945) we have presented the results obtained with two of the commonest weeds in barley on slightly acid soil derived from the lower greensand. These weeds, *Spergula arvensia* (spurrey) and *Matricaria inodora* (mayweed), were chosen for study because they were specially characteristic of the land in question. In the present paper the same methods have been applied to chickweed (*Stellaria media*). This is a weed which affects almost every field and garden in the country, flourishes and seeds as far north as Spitzbergen, and occurs over almost all the temperate parts of the world. Though not considered as a particularly vicious weed, yet it is a continual nuisance, and, especially if the ground is well manured either by artificial fertilizers or by farmyard manure, it will frequently smother the crop among which it is growing. It is not dominant on the soil at Woburn, but forms about 20% of the total weed herbage, this being increased up to 30% by the addition of mineral manures (Mann, 1939). It is more frequent on soil which has been limed: when the pH of the soil falls below 5·7, *S. media* quickly disappears, and on highly acid soil such as that where sulphate of ammonia has been used for many years without lime, it is entirely absent.

METHOD OF STUDY

As described in previous papers in this series, the experiments were conducted in conditions where the root space was constant but limited, where there was always sufficient water supply though the soil was well aerated, and where the nitrogen, phosphate and potash supply was enough for both the main crop of barley and for the weed. The root space available for each plant could be varied by altering the number of plants in the given space, but the other factors remained fixed during the experiments. Both barley and the weed were grown in earthenware pots 28 cm. in diameter and 25·5 cm. deep furnished with an upturned outlet near the bottom which enabled watering to be done without danger of loss and yet secured good aeration of the soil. The bottom of the pots was covered with coarse gravel to above the outlet. Each pot was then filled with 16·3 kg. of soil taken from one of the fields at Woburn where chickweed was common and vigorous. This gave about 20 cm. depth of soil (12·3 l.), or 14·2 l. including the gravel.

In previous papers we have shown (Mann & Barnes, 1947, 1949) that the addition of 1·25 g. nitrogen/pot of the present size (or 0·09 g. nitrogen/l. of root space) ensured a full yield of barley. A slightly larger amount was, however, added in the present case, in three instalments, the first on 1 April (0·75 g.), the second on 28 May (0·50 g.), and the third on 26 June (0·25 g.), so that at no time either the barley or the chickweed showed signs of nitrogen shortage. Barley was sown on 17 April and thinned to the proper number per pot on 2 May. Young seedling plants of chickweed taken from the field were planted on 16 April, and a few which failed were replaced on 24 April.

With barley grown alone, we have evidence that more than four plants per pot (154 sq.cm. of surface or 3·5 l. of root space per plant) gave very little increase in yield. In the case of chickweed, the thickness of planting which led to the most favourable growth was determined under conditions similar to those already studied for barley. Portions of seedling chickweed plants from the field were planted in the soil at spacings ranging from one plant per pot (616 sq.cm./plant) to eight plants per pot (77 sq.cm./plant), or a soil volume available per plant ranging from 14·2 to 1·8 l. The details of the growth obtained with each thickness of planting are shown in Table 1.

TABLE 1. *Effect of thickness of planting on yield of chickweed*

No. of chickweed plants per pot	Root space per plant (l.)	Wt. of air-dry chickweed (above ground) per pot (g.)	Wt. of air-dry chickweed roots per pot (g.)	Nitrogen in dry matter (above ground) (%)	Root space per 1 g. roots (l.)
1	14·2	71·7	5·5	1·06	2·58
2	7·1	75·7	6·0	1·18	2·36
4	3·5	78·7	18·5	1·24	0·77
6	2·4	72·0	14·3	1·31	0·99
8	1·8	67·6	13·3	1·30	1·07

The maximum growth per unit area is reached with four plants per pot, and no

greater production of either tops or roots is obtained by thicker sowing. The roots evidently did not fill the space available to them till at least four plants per pot were present, but with greater numbers than this the weight of roots tends to decrease as the number of plants becomes greater. On the other hand, as far as growth above the soil is concerned, the one plant is able to give nearly as great a weight of dry matter as any larger number, while with more than six plants per pot, there appears to be such competition between the chickweed plants as to reduce the total yield.

It is interesting to compare the relative amount of the above-ground portions and the roots of chickweed with the other annual weeds we have studied under similar conditions. With a concentration of six plants per pot, the proportion of roots to the whole plants in each case was: (1) chickweed 16·6%, (2) spurrey 10·2%, (3) mayweed 10·1%.

If the root development has any relationship to the effectiveness of the weed as a competitor, it would appear that chickweed is a more powerful competitor than either of the other two species mentioned.

Table 2 gives a more complete statement of the relative behaviour of these weeds, taken as a mean at all thicknesses of planting, in comparison with barley itself.

TABLE 2. *Comparison of several annual weeds with barley*

	Spurrey (1943)	Mayweed (1943)	Chickweed (1946)	Barley (1946)
Wt. of plants and roots per pot (g.)	68·3	80·0	86·3	117·4
Wt. of above-ground portions per pot (g.)	61·3	71·9	72·0	103·9
Nitrogen in above-ground portions per pot (mg.)	1174	1167	861	1057
Nitrogen in roots per pot (mg.)	101	150	142	169
Proportion of root wt. to total wt. (%)	10·2	10·1	16·5	11.5
Proportion of total nitrogen in roots (%)	7·9	11·4	14·1	13·8

The chief interest in Table 2 lies in the proportion of the plant foods supplied which is fixed in the roots and so withdrawn from the production of the above-ground organs. Mann & Barnes (1949) showed that in several couch or twitch grasses the nitrogen remaining in the roots was from one-third to one-half of the total nitrogen absorbed by the plants. In crop plants like barley, the proportion so locked up is far smaller. The weeds now considered are much more like barley in this respect, and hence should be far less injurious to crops among which they are grown than the twitch grasses which impound a greater amount of the fertilizing ingredients from the crops among which they grow.

COMPETITION BETWEEN BARLEY AND CHICKWEED (*STELLARIA MEDIA*)

In considering competition between barley and chickweed the factors studied have been: first, the effect of increasing the thickness of barley sowing when the weed

infestation remains constant, and, secondly, the effect of an increase in the amount of weed infestation when the stand of barley remains the same.

Weed infestation constant: barley thickness variable

In these experiments, there were six plants of chickweed per pot, and from one to eight plants of barley. The chickweed was transplanted from the field practically at the same time as the barley was sown (chickweed, 16 April; barley, 17 April). The results of their growing together are shown in Table 3.

TABLE 3. *Yield of barley and chickweed, with varying amounts of barley*

No. of plants per pot		Max. no. of barley shoots	Yield of barley per pot		Yield of chickweed per pot (air-dry) (g.)	Yield of roots	
Barley	Chickweed		Grain (g.)	Total (g.)		Barley (g.)	Chickweed (g.)
0	6	—	—	—	71·0	—	14·3
1	6	6	3·4	7·4	68·8	1·0	7·6
1	0	39	18·2	76·2	—	11·4	—
2	6	10	5·4	12·3	63·4	1·6	9·7
2	0	49	30·9	92·6	—	9·1	—
4	6	19	9·2	21·5	59·8	2·0	12·7
4	0	64	30·0	98·3	—	12·7	—
6	6	20	8·3	18·6	69·0	2·5	23·0
6	0	70	36·5	103·9	—	13·5	—
8	6	28	18·0	39·1	49·0	5·2	11·5
8	0	77	38·3	103·1	—	15·8	—

Table 3 shows what a very effective competitor chickweed may be with barley when the plants start almost at the same time, and to what extent a thick seeding of barley will reduce the effect of the competition of the weed. Table 4 shows the percentage reduction in the yield of barley owing to the presence of the chickweed, and of chickweed owing to the presence of the barley.

TABLE 4. *Percentage reduction in yield of barley owing to presence of chickweed and of chickweed owing to presence of barley*

No. of plants per pot		Percentage reduction in yield		
		Barley		Chickweed
Barley	Chickweed	Grain (%)	Total above-ground wt. (%)	Total above-ground wt. (%)
1	6	81·3	90·3	4·4
2	6	82·5	86·7	11·9
4	6	69·4	78·1	16·9
6	6	77·2	82·0	4·2
8	6	53·0	62·1	31·9

Despite some irregularity in the figures for six plants of each kind the general results are quite clear. The increased thickness of sowing of the barley greatly affects the competitive power of the crop over the weed. With the highest thickness of the barley (eight plants per pot, or 77 sq.cm. of surface per plant) the reduction in yield of barley in the presence of six plants of chickweed per pot (103 sq.cm. of surface per plant), though still great, is far less than when a single plant is competing with six plants of chickweed in the same area. On the other hand, chickweed is very much less affected by the presence of the barley. With one plant of barley to six plants of weed, there is merely a reduction in the latter of 4%, and it is only when the thickness of the barley is much increased that the growth of the weed is seriously affected. The persistence of this weed in the presence of barley is far greater than that of either spurrey or mayweed. The relative effect of these three weeds is shown in Table 5.

TABLE 5. *Comparison of chickweed with spurrey and mayweed as competitive weeds with barley*

No. of plants per pot		Percentage reduction in total produce					
		Chickweed		Spurrey		Mayweed	
Barley	Weed	Reduction in barley	Reduction in weed	Reduction in barley	Reduction in weed	Reduction in barley	Reduction in weed
1	6	90	4	36	85	61	70
2	6	87	12	33	83	42	76
4	6	78	17	10	93	35	79
6	6	82	4	5	94	22	85
8	6	62	32	Nil	94	20	87
	Mean	80	14	17	90	36	79

Table 5 shows that of the three annual weeds studied, chickweed is by far the most powerful competitor with barley when the conditions are favourable both for the barley and the weed, and spurrey very much the least powerful, mayweed lying between them. If the thickness of planting is increased, the effect of spurrey may be eliminated, that of mayweed may be reduced by half. With chickweed, even with the thickest planting of barley, the crop obtained is only about two-thirds that when the weed is absent. The technique we have used seems suitable for measuring the relative competitive power of the several weeds when the conditions are favourable for both weed and crop.

Barley plant constant: weed infestation varying

In examining the result of increasing the number of chickweed plants on the vigour and development of a sparse barley crop, a fixed number of two barley plants per pot were sown, thus allowing 7·1 l. of root space per plant. The number of chickweed plants varied from none to eight. As before, the barley and the weed were planted at substantially the same time. The results are shown in Table 6.

TABLE 6. *Yield of barley and chickweed with varying amount of the weed*

No. of plants per pot		Max no. of barley shoots per pot	Yield of barley per pot		Yield of chickweed per pot (above ground) (g.)	Yield of roots per pot	
Barley	Chick-weed		Grain (g.)	Total produce (g.)		Barley (g.)	Weed (g.)
2	0	49	30·9	92·6	—	9·1	—
2	1	?	10·9	25·9	90·1	3·4	19·7
0	1	—	—	—	71·7	—	15·5
2	2	21	18·9	46·2	44·5	6·2	8·5
0	2	—	—	—	75·7	—	6.0
2	4	10	8·9	20·0	62·3	2·7	11·7
0	4	—	—	—	78·7	—	18·5
2	6	11	6·4	14·5	64·1	1·9	6·7
0	6	—	—	—	72·0	—	14·3
2	8	7	3·7	8·8	66·7	1·2	17·2
0	8	—	—	—	67·6	—	13·3

The percentage reduction in the yield of barley owing to the presence of chickweed and of chickweed owing to the presence of barley is shown in Table 7, which relates only to the portion of the plants above ground.

TABLE 7. *Percentage reduction in yield of barley owing to presence of chickweed and of chickweed owing to presence of barley*

No. of plants per pot		Percentage reduction in yield		
		Barley		Chickweed
Barley	Chickweed	Grain	Total above-ground wt.	Total above-ground wt.
2	1	64·7	72·0	−25·7 (increase)
2	2	38·8	50·1	41·2
2	4	71·2	78·4	20·8
2	6	79·3	84·3	11·0
2	8	88·0	90·5	1·3

In a sparse crop of barley, the increase of weed rapidly smothers the crop, and when the number of chickweed plants outnumbers that of barley, the latter almost disappears, while the chickweed is almost as luxuriant as when no barley is present. We are not sure that the increase of the chickweed consequent on the presence of a small amount of barley is a real one, but it is clear that the barley is more affected by the presence of chickweed than is the latter by the presence of barley.

It is interesting to compare the effect with that of the other annual weeds that we have studied, namely, spurrey and mayweed. If grown in a sparse crop of barley, with all other conditions favourable, the power of chickweed to smother a crop of barley is far greater than that of either of the other two weeds. Table 8 gives the

yield per pot with a constant amount of barley but with gradually increasing proportions of weed. With the spurrey and the mayweed, there were four plants of barley per pot, and the stubbles were not weighed; with chickweed there were two plants of barley and the stubbles were weighed and included in the produce given.

TABLE 8. *Comparison of chickweed with spurrey and mayweed as competitive weeds with barley*

No. of plants per pot		Spurrey Total wt. per pot		Mayweed Total wt. per pot		Chickweed Total wt. per pot	
Barley	Weed	Barley (g.)	Spurrey (g.)	Barley (g.)	Mayweed (g.)	Barley (g.)	Chickweed (g.)
2 or 4	1	92·0	2·0	95·8	5·8	?	?
2 or 4	2	89·5	4·5 (?)	95·4	4·2	46·2	44·5
2 or 4	4	90·3	5·6	95·8	7·1	20·0	62·3
2 or 4	6	91·0	6·7	82·6	17·8	14·5	64·1
2 or 4	8	85·0	10·6	79·5	19·2	8·8	66·7

The chickweed, it will be seen, has almost eliminated the barley when it is in the proportion of eight plants to two of barley, while at no thickness of sowing have either of the other two weeds been able to do more than reduce the yield of barley by more than about 8% with spurrey and 17% with mayweed. Chickweed at eight plants to two of barley has decreased the yield of the crop by 81% from that with two plants of the weed to two plants of barley. It must be remembered that the conditions have been arranged so that neither the crop nor the weed shall be under any stress for either water or plant food. If this were not the case and there was competition for an insufficient amount of either water or nitrogen, the situation might be quite different, but this situation is not studied in the present experiment.

METHOD OF INTERACTION BETWEEN BARLEY AND CHICKWEED

A careful examination of the root systems of the two plants in mixed cultures does not show any antagonistic relationship, and it seems as if there was simply competition for the space available for the root development. The roots of the barley and the weed occupy essentially the same levels in the soil. The primary roots of the barley, however, tend to go straight down and to give secondary roots coming off from the main roots at right angles. On the other hand, the chickweed roots are more winding and its secondaries arise at an acute angle from the main roots, and are much softer than the rather harsh barley roots. The finest rootlets of the chickweed seem to break off very much more easily than those of the barley.

If we consider the competition as for root space only, it would seem as if the relative growth of the two plants would be determined solely by the speed at which the roots develop and fill the space, and the plant whose roots develop the more rapidly would be an even more effective competitor with additional plants of its own kind than with the other plants among which it is grown. Now there is evidence that the growth of

chickweed roots in a favourable medium is more rapid than those of barley, and hence it would appear probable that when there is any serious root congestion the presence of the weed would be a greater hindrance to the growth either of barley or of additional weed plants than an increase in the number of barley plants. Table 9 shows the relative reduction of produce of two barley plants per pot due to competition with two, four or six weed plants as against a similar increase in the number of barley plants.

TABLE 9. *Comparative reduction in yield of barley and chickweed due to increase in the number of barley or weed plants*

	Percentage reduction due to additional barley	Percentage reduction due to additional weed plants
A. Reduction in produce of 2 barley plants		
With 2 additional barley or 2 weed plants per pot	46·9	50·1
With 4 additional barley or 4 weed plants per pot	62·6	78·4
With 6 additional barley or 6 weed plants per pot	72·2	86·7
B. Reduction in weed produce of 2, 4 or 6 weed plants		
2 weeds with 2 barley or 2 additional weed plants per pot	41·2	48·0
4 weeds with 2 barley or 2 additional weed plants per pot	20·8	39·1
6 weeds with 2 barley or 2 additional weed plants per pot	10·8	29·6

With a limited congestion of roots in the soil, there is little difference between the power of additional plants of barley or chickweed to reduce the growth of either the one or the other. But when the congestion is greater, a specified number of weed units is considerably more effective in reducing the yield of barley than a corresponding increase in units of the barley itself. A noticeable point, however, under these conditions is that an increase in the number of the weed units tends to reduce the growth of the barley more than it lowers the yield of the weeds themselves. The effect is still more notable in the total amount of weed produced, for here, with great congestion of weed roots in the soil, a further increase in the number of weed plants affects the total production of weed more than the addition of a similar number of units of barley. This is in accordance with the hypothesis already suggested that, under conditions of root congestion, the competitive power of a plant depends largely on the relative rapidity of growth of the roots of the cultivated plant and the weed.

DISTRIBUTION OF NITROGEN BETWEEN BARLEY AND CHICKWEED

We have already stated that in these experiments it was arranged that there should be always enough nitrogen to provide for the needs of both plants. If more manurial nitrogen is added, it only, so far as the barley is concerned, increases the nitrogen in

the plant and does not increase the yield. The experiments described below deal with the partition of the available nitrogen between the barley and the weed.

In the first place, it is clear that the barley grown alone is capable of absorbing and utilizing more nitrogen than either the chickweed grown alone or any mixture of the two plants. Out of 1500 mg. of nitrogen added as sulphate of ammonia in addition to that already supplied by the soil, the following were the mean amounts of nitrogen recovered in each case, including that contained in the roots of the plants concerned:

Barley grown alone	1222 mg. nitrogen per pot
Chickweed grown alone	910 mg. nitrogen per pot
Barley and chickweed (barley constant) ...	990 mg. nitrogen per pot
Barley and chickweed (weed constant) ...	973 mg. nitrogen per pot

The excess nitrogen contained in the barley crop seems, however, almost entirely to appear as a higher percentage of nitrogen in both grain and straw over that in the grain and straw when they are grown with chickweed. With the weed the percentage of nitrogen in the plant seems little affected by the presence or absence of the barley. The figures are given in Table 10.

TABLE 10. *Nitrogen in barley and chickweed when grown alone or together*

	Nitrogen: Percentage in dry matter			
	Barley grown alone	Barley grown with weed	Chickweed alone	Chickweed with barley
Grain	2·34	1·21	—	—
Straw and stubble	0·85	0·33	—	—
Roots	1·40	—	1·06	—
Above-ground portion	—	—	1·22	1·25

In the second place, the proportion of the nitrogen taken up by the plants which is contained in the above-ground portions is not widely different in the barley crop and in the weed, being 86·9% in barley and 88·5% in chickweed. This compares with 92·2% with spurrey and 88·4% with mayweed when grown under similar conditions in 1943.

A third point which appears is that at all levels of admixture, each plant of chickweed is able to capture a much larger amount of nitrogen than is each plant of barley. We can only give data for the above-ground portions of each plant, but Table 11 shows the amount of nitrogen found in this portion at all thicknesses of sowing.

In the presence of sufficient nitrogen for both plants, it is clear that under the mixed conditions the chickweed has the capacity of capturing far more per plant than has the barley. This is not due to any innate capacity to absorb nitrogen, for when grown alone, barley is able to take up more nitrogen per plant than is chickweed. But when grown together, it is clear that the weed can absorb nitrogen more readily than barley. This would suggest that when nitrogen is deficient or where there is a nitrogen stress, however caused, barley is likely to suffer more than the weed.

TABLE 11. *Nitrogen absorbed per plant of barley and chickweed in mixed culture*

	No. of plants per pot		Nitrogen found in above-ground portions of one plant	
	Barley	Chickweed	Barley (mg.)	Chickweed (mg.)
I.	1	6	46	134
	2	6	40	129
	4	6	33	119
	6	6	18	116
	8	6	31	101
II.	2	1	75	862
	2	2	157	242
	2	4	60	188
	2	6	42	121
	2	8	28	93

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STUDIES IN PLANT NUTRITION

II. FURTHER STUDIES OF THE EFFECT OF SOME ORGANIC SUPPLEMENTS ON THE GROWTH OF PLANTS IN SAND CULTURE

By H. E. STREET

Department of Botany, The University, Nottingham

The addition of an aqueous extract of leaf mould to lettuce and radish plants, grown in sand with inorganic culture solutions, caused significant increases in fresh and dry weights. An aqueous solution of a yeast extract caused significant, though smaller, increases in fresh and dry weights of radish plants. An aqueous extract of 'bacterized' peat had no significant effect on the growth of radish and was toxic to lettuce. The organic supplements had no significant effect on oats grown under similar conditions.

Neither the beneficial nor the toxic effects of the organic supplements can be satisfactorily explained on their content of auxins active in the *Avena* test.

The present status of our knowledge of the role of soluble soil organic matter in the nutrition of higher plants has been reviewed previously (Chesters & Street, 1948). The present paper describes further sand-culture experiments in which an examination has been made of the effects on plant growth and development of adding certain organic supplements to complete mineral culture solutions.

RADISH: VARIETY TURNIP RED, EXTRA EARLY, SHORT-TOP FORCING

Experimental

Seed boxes

The seed boxes used were of internal dimensions $12 \times 9 \times 4$ in., and contained water-washed Garsides 2L silica sand. Each box was coated with two applications of Bituros solution, and the drainage holes were plugged with glass-wool.

Culture solution

The culture solution was prepared, according to the formula given by Woodman (1940), from the following salts: $(NH_4)_2SO_4$, $MgSO_4.7H_2O$, $Ca(NO_3)_2.4H_2O$, $NaNO_3$, KNO_3, KH_2PO_4 and contained the following p.p.m. of the cultural elements: nitrate-N 24·5, ammonium-N 8·46, S 13·04, P 8·19, K 22·44, Mg 5·05, Ca 9·03, Na 17·5. To each litre of this solution was added 1 ml. of the concentrated micronutrient solution, previously described (Chesters & Street, 1948). The complete culture solution was prepared each week by dilution of concentrates with City of Nottingham tap water. The pH of the culture solution was 6·4–6·6.

Nutrient treatments

Treatment I. Complete culture solution + 10 ml./l. of a solution of sodium nitrate, containing 6·65 mg. N/10 ml.

Treatment M. Complete culture solution + 10 ml./l. of an aqueous extract of leaf mould, containing 6·65 mg. N/10 ml. The leaf-mould extract was prepared by the procedure previously described (Chesters & Street, 1948), but from a new standardized bulk sample of mould.

Treatment Y. Complete culture solution + 10 ml./l. of an aqueous solution of yeast extract (Yeastrel), containing 6·65 mg. N/10 ml.

Treatment P. Complete culture solution + 10 ml./l. of an aqueous extract of a commercial sample of bacterized peat, containing 6·65 mg. N/10 ml. The extract of bacterized peat was prepared by the procedure used in preparing the leaf-mould extract. The extract was prepared in bulk and stored at −20° C. until required.

Plan of experiments

Experiment 1

Arrangement. Four randomized blocks, each of two boxes for treatments I and Y.

Time-table of operations:

2. v. 47. Sand saturated with appropriate treatment solution at half-strength.

3. v. 47. Each box set with twenty-eight seeds (four rows of seven seeds) of radish, variety Turnip Red, Extra Early, Short-Top Forcing.

4. v. 47 and 7. v. 47. Applied 500 ml. treatment solution (half-strength) per box.

9. v. 47 to 17. vi. 47. Applied three times per week 500 ml. treatment solution per box.

30. v. 47. Seedlings thinned to leave twelve per box.

17. vi. 47. Plants harvested.

When harvested all plants were healthy and the storage roots sound and of good colour. The plants receiving the Y treatment were larger and the leaves deeper green by 17 May. Swelling of the roots was earlier in plants receiving the Y treatment. At harvest the fresh and dry weights of the shoot and root systems of each batch of plants were separately determined. The storage organ ('radish'), derived from the base of the hypocotyl, was regarded as part of the root system. The dry weights were determined by the method previously described (Street, Kenyon & Watson, 1946), any sand adhering to the dried tissues being removed.

Experiment 2

Arrangement. Six randomized blocks each of four boxes for treatments I, M, Y and P. The experiment was conducted on similar lines to Exp. 1.

Time-table of operations:

20. iv. 48. Saturation of sand with treatment solution.

21. iv. 48. Seeds set forty per box (four rows of ten seeds).

10. v. 48. Seedlings thinned to sixteen per box.

15. vi. 48. Plants harvested.

Results

The results of the two experiments, taken together, indicate that both the M treatment and the Y treatment caused significant stimulation of the growth of the radish, assessed on both a fresh- and a dry-weight basis. The P treatment has no deleterious effect upon the growth of the radish. Significant differences in shoot/root ratios, or in moisture contents of plants receiving the different treatments, have not been established (Table 2).

Table 1. *Results obtained with radish, variety Turnip Red, Extra Early, Short-Top Forcing, in Exp.* 1 (*harvest* 17 *June* 1947). *Mean fresh weights* (F.), *and dry weights* (D.), *for single plants and plant organs in* g.

W. = whole plant; S. = shoot; R. = root system.

Description of data	Treatment means (g.) I	Y	Standard errors
F.W.	4·39	5·88	0·25
F.S.	1·72	2·52	0·18
F.R.	2·67	3·36	0·15
D.S.	0·151	0·212	0·014
D.R.	0·239	0·345	0·021

Table 2. *Results obtained with radish, variety Turnip Red, Extra Early, Short-Top Forcing, in Exp.* 2 (*harvest* 15 *June* 1948). *Mean fresh weights* (F.), *and dry weights* (D.), *for single plants and plant organs in* g.

W. = whole plant; S. = shoot; R. = root system; Rad. = 'radish' with fibrous roots removed.

Description of data	Treatment means (g.) I	M	Y	P	Standard errors
F.W.	3·75	5·03	4·26	3·96	0·11
F.S.	1.26	1·77	1·44	1·36	0·03
F.Rad.	1·88	2·60	2·13	1·95	0·10
D.S.	0·103	0·140	0·112	0·109	0·004
D.Rad.	0·160	0·204	0·176	0·166	0·007
F.S./F.R.	0·506	0·543	0·511	0·523	
D.S./D.Rad.	0·644	0·683	0·636	0·651	F. test
% moisture, S.	92·1	92·1	92·4	91·9	negative
% moisture, Rad.	91·5	91·9	91·5	91·3	

SPRING OATS

Experimental

Culture vessels

The culture vessels used were 2 gal. glazed earthenware Doulton pots ($8\frac{1}{2}$ in. internal diameter, $10\frac{1}{2}$ in. deep). Each pot had at its base a lateral drainage tubulure, into which was inserted a plug of glass-wool and a rubber stopper carrying a glass

drainage tube. The pots were each filled with water-washed Garside's 2 L silica sand. They were kept in an unheated greenhouse.

Culture solutions

Two culture solutions were prepared to the formulae shown below:

	Molar concentrations	
	Culture solution no. 1	Culture solution no. 2
Potassium nitrate, KNO_3	0·0044	0·0042
Magnesium sulphate, $MgSO_4.7H_2O$	0·0024	0·0024
Potassium dihydrogen phosphate, KH_2PO_4	0·0063	0·0063
Ammonium sulphate, $(NH_4)_2SO_4$	0·0042	0·0014
Calcium nitrate $Ca(NO_3)_2.4H_2O$	0·0044	0·0044

Culture solution no. 1 is solution $T_3R_3C_3$ of the modified Tottingham series (Jones & Shive, 1921). Culture solution no. 2 is a modification with enhanced potassium content and with a higher nitrate:ammonium ratio (Sessions & Shive, 1933; Stahl & Shive, 1933). To each litre of both culture solutions was added 1 ml./l. of the concentrated micro-nutrient solution previously described (Chesters & Street, 1948). The solutions were prepared each week by dilution of convenient concentrates with tap water. Solutions nos. 1 and 2, together with any of the treatment additions listed below, had initial pH values within the range 5·35–5·60. The solution draining from the culture vessels was frequently tested and found to be slightly more acid with a pH between 5·3 and 4·8. Solution no. 1 was used to prepare the treatment solutions applied during the first thirty days from setting of the grain, and was then replaced by solution no. 2.

Nutrient treatments

The treatment solutions employed were the same as for the radish experiment, except that the standardized bulk sample of leaf mould was that used in the 1947 lettuce experiment described in a previous paper (Chesters & Street, 1948).

Plan of experiments

Experiment 3

Arrangement. Nine randomized blocks, each of three pots for treatment I, M and Y.

Time-table of operations:

9. v. 47. Sand saturated with appropriate treatment solution (prepared from solution no. 1).

16. v. 47 to 20. ix. 47. Applied three times per week 500 ml. treatment solution per pot (prepared from solution no. 1 up to 8 June and from solution no. 2 from 10 June to 20 September).

10. vi. 47. Commenced flushing the pots with 2 l. of water at 10-day intervals.

7. vii. 47. Commenced fortnightly nicotine fumigations.

14. vii. 47. Plants sprayed with colloidal sulphur preparation. Effected a successful check of an attack of *Erysiphe graminis* noted on a few plants.

10. v. 47. Each pot set with sixteen even-sized dehulled grains of S. 221 Spring Oats (supplied from Welsh Plant Breeding Station, Penglais, Aberystwyth).

21. v. 47. Seedlings thinned to eleven per pot.

9. vi. 47. Seedlings thinned to six per pot.

30. vi. 47. Seedlings thinned to three per pot.

22 and 23. ix. 47. Plants harvested.

Experiment 4

Arrangement. Nine randomized blocks, each of five pots. Data for only two treatments, I and P, are presented in this paper.

Time-table of operations:

19. iv. 48. Sand saturated with appropriate treatment solution (prepared from solution no. 1).

22. iv. 48 to 21. viii. 48. Applied three times per week 500 ml. treatment solution per pot (prepared from solution no. 1 up to 17 May and from solution no. 2 from 19 May to 21 August).

19. v. 48. Commenced flushing the pots with 2 l. of water at 10-day intervals.

7. vii. 48. Commenced fortnightly nicotine fumigations.

20. iv. 48. Each pot set with 10 grains of 01747/10/7 Spring Oats (supplied from Welsh Plant Breeding Station).

5. v. 48. Seedlings thinned to five per pot.

23–25. viii. 48. Plants harvested.

Results

Statistical analysis of these data showed no significant differences between the treatment means. The uniformity of results in Exp. 3 is striking. Exp. 4 suggests

Table 3. *Results obtained with S. 221 Spring Oats in Exp. 3. Mean fresh weights* (F.), *and dry weights* (D.), *for single plants and plant organs in* g.

W. = whole plant; S. = shoot; Inf. = mature panicles; R. = root.

Harvest type and harvest dates	Description of data	Treatment means (g.) I	M	Y
Seedling harvest 9 June 1947	F.W.	3·55	3·24	3·48
	D.W.	0·338	0·314	0·310
Seedling harvest 30 June 1947	F.W.	29·3	29·6	24·9
	D.S.	2·48	2·50	2·04
Main harvest 22 Sept. 1947	F.W.	94·5	89·0	83·0
	D.W.	19·5	18·4	18·1
	D.S.	17·7	16·6	16·5
	D.R.	1·84	1·67	1·60
	D.Inf.	4·50	4·43	4·18

Table 4. *Data obtained for S. 221 Spring Oats in Exp* 3

Date	Plants examined per treatment	Description of data	Treatment means I	M	Y
9 June 1947	99	Height of shoot (cm.)	54·0	53·8	53·2
		Leaves per plant	8·7	8·1	8·2
		Tillers per plant	1·4	1·2	1·2
30 June 1947	54	Height of shoot (cm.)	122	122	124
		Tillers per plant	3·6	3·3	3·2
24 July 1947	27	Height of shoot (cm.)	153	153	157
		Tillers per plant	11·1	10·4	10·4
		Fertile tillers per plant	4·0	4·3	4·0
22 Sept. 1947	27	Tillers per plant	48	47	42
		Fertile tillers per plant	44	44	39
		Fully mature panicles per plant	11	12	10

Table 5. *Results obtained with* 01747/10/7 *Spring Oats in Exp.* 4

F. = mean fresh weight per plant or plant organ. D. = mean dry weight per plant or plant organ. W. = whole plant; S. = shoot; R. = root. * = 45 plants examined per treatment.

Description of data	Treatment means I	P
F.W. (g.)	107·8	94·5
D.W. (g.)	15·02	13·80
F.S. (g.)	80·5	68·3
D.S. (g.)	13·9	12·7
F.R. (g.)	27·3	26·2
D.R. (g.)	1·12	1·10
*Height of shoot (cm.)	152	162
*Tillers per plant	10	10
*Fertile tillers per plant	5	7
*Fully mature panicles per plant	3	3

that the effect of the P treatment was slightly deleterious, but there was no evidence that the root growth was checked by the P treatment as happened in the lettuce experiments described later in this paper. The excellent growth and development of the oat plants in both experiments make it improbable that their uniformity was due to the operation of unfavourable limiting factors. It must therefore be concluded that the Spring Oats are unaffected by application of these organic supplements at concentrations which influence significantly the growth and development of both lettuce and radish.

LETTUCE: VARIETY MAY KING IMPROVED

Experimental

The culture vessels used were 7 in. diameter plant pots covered internally with a double coat of 'Bituros' solution and filled with Garside's 2L silica sand. The culture solution was identical with that used in our previous work with lettuce (Chesters & Street, 1948).

Nutrient treatments

Treatment I. As for radish + 10 ml./l. 0·5% ethyl alcohol.

Treatment M. As for radish + 10 ml./l. 0·5% ethyl alcohol.

The standardized bulk sample of mould used in the present work was different from that used in 1947 (Chesters & Street, 1948).

Treatment ME. Complete culture solution + 10 ml./l. of the solution of sodium nitrate used for treatment I + 10 ml./l. of solution ME. Solution ME was prepared thus: 1 l. of the aqueous extract of leaf mould was acidified with 5 ml. of glacial acetic acid and then extracted successively and in a separating funnel with 200 ml. followed by 4 × 100 ml. portions of ethyl ether. The combined ether layers were washed with 25 ml. of water and then evaporated to small bulk (about 10 ml.) at 25° C. *in vacuo*. The residue was taken up in 25 ml. 95% ethyl alcohol and then evaporated to dryness at 30° C. *in vacuo*. The residue was dissolved by warming in 5 ml. absolute ethyl alcohol and then diluted with warm distilled water, cooled and adjusted with cold distilled water to 1 l. to give solution ME. This was stored in a closed container in the refrigerator.

Treatment P. As for radish + 10 ml./l. of 0·5% ethyl alcohol.

Treatment PE. Complete culture solution + 10 ml./l. of the solution of sodium nitrate used for treatment I + 10 ml./l. of solution PE. Solution PE was prepared from the aqueous extract of 'bacterized' peat by the same procedure as that used to prepare solution ME.

Plan of experiment

Experiment 5

Arrangement. Twelve randomized blocks each of five pots.

Time-table of operations:

4. v. 48. Sand of each pot saturated with appropriate treatment solution at half-strength.

18. v. 48 to 24. vii. 48. Applied three times per week and according to weather conditions, either 250 or 500 ml. of treatment solution per pot.

26. vii. 48 to 8. viii. 48. Applied three times per week 500 ml. treatment solution per pot.

5. v. 48. Each pot set with twelve seeds of lettuce, variety May King Improved (Watkins and Simpson).

20. v. 48. Seedlings thinned to four per pot.

9. vi. 48. Harvested three seedlings per pot.

8. vii. 48. Harvested six blocks.

10. viii. 48. Harvested remaining six blocks.

When harvested on 8 August no plants were discoloured, and, except for some leaf scorch affecting the P treatment plants and to a less extent the PE treatment plants, all were healthy and in flower. Bolting began on 8 July and was evident in all plants

by 22 July. The onset of bolting was independent of treatment, but in each block the M plants opened flowers in advance of other plants of their replicate.

Results

In Table 6, L. represents leaves of the main shoot from sand level to the basal part of the inflorescence, and St. represents the main stem and inflorescence axes including the leaves of the upper part of the main shoot, and of the axillary shoots and the flowers and the flower buds.

Table 6. *Results obtained with lettuce, variety May King Improved, in Exp.* 5. *Mean fresh weights* (F.), *and dry weights* (D.), *for single plants and plant organs in* g.

W. = whole plant; S. = shoot; L. = leaves of main shoot; St. = main stem and inflorescence; R. = root.

Harvest no.	Description of data	Treatment means (g.) I	M	ME	P	PE	Standard errors
1	F.W.	1·66	2·17	1·59	1·52	1·44	0·10
	F.S.	1·53	1·97	1·45	1·38	1·30	0·08
	D.S.	0·100	0·132	0·098	0·090	0·087	0·005
2	F.W.	62·0	92·5	63·4	31·7	59·3	7·0
	F.S.	43·3	58·0	41·2	27·7	38·4	2·3
	F.R.	18·7	34·4	21·9	4·0	20·9	2·9
	D.S.	2·46	3·78	2·91	1·53	2·38	0·21
	D.R.	1·54	2·62	1·55	0·26	1·39	0·19
3	F.W.	158·2	171·3	171·4	134·6	153·0	3·0
	D.W.	13·59	17·18	15·01	11·52	13·24	0·53
	F.S.	122·0	120·5	122·1	108·8	117·6	2·8
	D.S.	10·64	12·56	10·61	9·54	10·48	0·44
	F.L.	60·7	57·9	64·0	52·0	57·8	1·7
	D.L.	4·54	4·78	4·85	3·92	4·38	0·14
	F.St.	61·3	62·6	58·1	56·6	59·8	1·9
	D.St.	6·10	7·78	5·76	5·62	6·10	0·45
	F.R.	36·2	50·8	49·3	25·8	35·4	2·1
	D.R.	2·95	4·62	4·40	1·98	2·76	0·21

At harvests nos. 1 and 2 plants receiving the M treatment had significantly greater fresh and dry weights than those receiving the other treatments. At the final harvest (no. 3), plants receiving treatment M had greater dry weights of shoots and greater fresh and dry weights of roots than those receiving other treatments; otherwise treatments I, M, ME and PE gave similar yields. The ME treatment, too, produced greater root weights than treatments I, PE and P. Plants receiving treatment P were smaller in all respects but especially in root weight. No similar retardation of root growth resulted from the PE treatment.

The results recorded in Table 6 are similar to those previously obtained with lettuce during the 1947 season (Chesters & Street, 1948). Exact agreement is not to be expected between plants grown under different climatic conditions (the summers of 1947 and 1948) which altered the time of onset of 'bolting' (64–66 days after sowing in 1947 and 76–79 days after sowing in 1948) and the general growth rate of

the plants.* In particular, the stimulation of root development by the M treatment recorded in 1948 was more marked than that observed in the 1947 experiment and, as a result, the increased shoot/root ratios recorded in 1947 were not apparent in the present work. It should be pointed out that a new bulk sample of leaf mould was employed, and therefore the present M treatment cannot be regarded as directly comparable with that used in the 1947 experiment.

DISCUSSION

The addition of an aqueous extract of leaf mould to the complete mineral nutrient solution increased the fresh and dry weights of radish and lettuce. Yeast extract produced a smaller but still significant stimulation of the growth of radish but was slightly deleterious to lettuce (Chesters & Street, 1948). The aqueous extract of a commercial sample of 'bacterized' peat did not affect radish growth but was toxic to lettuce. The oat plants were unaffected by the organic supplements. The insensitivity of the monocotyledon, oats, and the sensitivity of the two dicotyledons, radish and lettuce, suggested the possibility that the auxin content of the organic supplements might be important. Succulent dicotyledons are not only more sensitive to the synthetic auxin-like substances developed as selective weed killers but apparently absorb, translocate and accumulate synthetic hormones more rapidly than do some monocotyledons (Mitchell, Wood, Wolfe & Irving, 1947). The enhanced root development in lettuce receiving the leaf-mould treatment also seemed to simulate the results obtained by various workers in response to application of naphthalene acetamide at low concentrations to plants in soil and sand cultures (Mitchell & Stewart, 1939; Laude, 1941; Hamner, 1942).

If our results are explicable in terms of such an auxin response, lettuce should be more sensitive to auxin than radish, and our organic supplements should, in order of increasing auxin activity, fall into the sequence: leaf mould, yeast extract, 'bacterized' peat. Dr E. S. J. Hatcher of the East Malling Research Station has kindly examined our aqueous extracts of leaf mould, yeast extract and 'bacterized' peat for activity in the *Avena* test and has found that none of the treatment solutions here employed has auxin values exceeding 8×10^{-5} mg. indoleacetic acid per litre. Hamence (1945) has determined the total auxin and free β-indolyl-acetic acid contents of organic manures. He reported the total auxin per 100 g. dry matter of a sample of leaf mould as 0·007 mg. and of a sample of peat as 0·08 mg. In both cases only an inappreciable amount of the total auxin was in the form of free β-indolyl-acetic acid. Furthermore, Templeman & Marmoy (1940) have failed to obtain any stimulation of the growth of lettuce in soil and sand cultures from applications of sodium α-naphthylacetate, at concentrations ranging from 0·005 to 33·3 mg./l., and

* Mean fresh weight per plant in g. (F.W.):

	Years and days after sowing	
Treatment	1947 (62 days)	1948 (65 days)
I	87·9 g.	62·0 g.
M	115.0 g.	92.5 g.

Stewart & Hamner (1942) have obtained similar negative results from the treatment of radish seed with a wide range of concentrations of a number of synthetic auxins. A series of unpublished sand-culture experiments undertaken by the author with both lettuce and radish have failed to show any significant stimulation of growth from applications, in the nutrient solution, of β-indolyl-acetic acid, α-naphthylacetic acid or naphthalene acetamide at concentrations ranging from 0·1 to 2·5 p.p.m. It seems improbable, therefore, that our results are to be explained as responses to growth-regulating substances showing activity in the *Avena* test. The absence of toxic symptoms in the lettuce plants receiving the PE treatment also discounts the suggestion that the toxicity of 'bacterized peat' is related to its auxin constituents. The results obtained with the ME treatment do, however, indicate that some of the stimulatory activity of leaf mould is contained in the ether-soluble acid fraction of the extract.

The author wishes to express his sincere thanks to Dr E. S. J. Hatcher for carrying out the *Avena* tests; to Mr R. Brook and Mr C. S. Lowe for their voluntary technical assistance; and to Prof. C. G. C. Chesters for his constant help and encouragement.

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THE EFFECT OF CHARCOAL ON THE GROWTH OF LEGUMINOUS PLANTS IN SAND CULTURE

By J. T. VANTSIS and G. BOND*
Department of Botany, University of Glasgow

The addition of small proportions (0·5–2·0%) of activated charcoal to the rooting medium of inoculated peas in nitrogen-free sand culture resulted in marked increases in dry weight of the plants and in nitrogen fixation. Wood charcoal in larger proportions had a similar effect, while animal charcoal severely inhibited growth. The number of nodules was greatly reduced in the presence of activated charcoal, but such nodules as formed were much larger and the nodule tissues per unit weight were more active in nitrogen fixation. Activated charcoal also led to an increase in dry weight of non-inoculated peas supplied with inorganic combined nitrogen. It is tentatively suggested that these favourable effects arise from the adsorption by the charcoal of harmful excretions from roots or micro-organisms or of excess nutrients, and from the maintenance of a more favourable pH in the rooting medium. The examination of barley intersown with the peas, and the results of Kjeldahl analyses on the rooting media, provided no evidence that the enhanced fixation in the presence of activated charcoal was attended by any considerable excretion of fixed nitrogen.

Introduction

Although the addition of charcoal to rooting media is a frequent practice in horticulture, the literature yields relatively few accounts of actual investigations of the effect and mode of action of the charcoal. Prianishnikov & Domontovitch (1926) report an experiment in which the addition of charcoal to sand cultures of cereals increased the yield in some cases by as much as three times, the suggested explanation being that the charcoal by its adsorptive properties prevented the development of an unfavourable pH. Zinzadzé (1932) found that the addition of activated charcoal to water cultures maintained at various pH levels by buffers resulted in improved plant growth, the increase in yield amounting in some cases to 30%. The beneficial effect was considered to be due chiefly to the adsorption of toxins by the charcoal, other possibilities mentioned being that the charcoal catalyzed certain beneficial oxidations or supplied minor elements to the medium. Papadakis (1941), commenting on the results of Zinzadzé and on the beneficial effect observed by Holynski (1928)† of the addition of charcoal to soil cultures, also ascribes the results to adsorption by the charcoal of toxins and to the oxidation of toxins under the influence of the charcoal.

Gukova & Butkevich (1941) reported that the addition of 6% of coarsely powdered

* The experiments forming the basis of this paper were carried out by J.T.V. during his tenure of a British Council Scholarship, and form part of a thesis submitted by him and approved by the University of Glasgow for the degree of Ph.D. The work was performed under the supervision of the second-named author and was to some extent facilitated by a grant made to him by the Agricultural Research Council.

† The authors have been unable to consult this paper.

charcoal (presumably ordinary wood charcoal) to sand cultures of inoculated Soya bean resulted in the dry weights of the plants and of the nodules being approximately doubled, rather similar effects being produced by the addition of peat instead of charcoal. The charcoal was considered to be without effect on uninoculated plants supplied with combined nitrogen; the fact that the growth of these plants was actually reduced in the presence of charcoal was attributed to a deficiency of mineral nutrients in these particular cultures. With peat the growth of uninoculated plants was unaffected. The striking benefit to the nodulated plants was attributed to the improved aeration which the charcoal was thought to produce.

The finding (Rothamsted Experiment Station Report, 1946, p. 91) that the addition of charcoal to the soil considerably reduced the incidence of clover sickness may be noted, and also the report by Tincker (1947) of the beneficial effect (attributed to improved aeration) of charcoal on the growth of *Lilium* spp.

The present paper records observations on the effect of the addition of charcoal to sand cultures of a leguminous plant (pea) with particular reference to the work of Gukova & Butkevich.

Materials and methods

Ordinary unglazed pots, or in some cases glazed pots, were used as containers for the plant cultures. Two types of near-white sand were employed, namely, a relatively coarse horticultural sand and a finer industrial sand. Mechanical analyses of the sands, which will subsequently be referred to as 'coarse' and 'fine' sand respectively, are as follows:

Particle size (mm.)	Horticultural sand (%)	Industrial sand (%)
>2·4	1·6	0·0
1·2–2·4	6·5	0·0
0·6–1·2	34·0	0·2
0·3–0·6	53·8	29·3
0·15–0·3	3·3	62·0
<0·15	0·8	8·5

The sands showed an initial total nitrogen content varying in different consignments from 11 to 17 mg./kg. Though the evidence suggested that for the most part this nitrogen was unavailable to plants, it was appreciated, in connexion with the detection of excretion of fixed nitrogen (see later), that the use of the sand with this nitrogen unremoved meant that the experiments could serve to provide clear evidence of extensive excretion only.

Three types of charcoal were employed, namely, wood charcoal, activated charcoal (to which most of the experiments relate) and animal charcoal. The wood charcoal consisted of granules 0·5–3 mm. in diameter. Two types of activated charcoal, powdered and granular, were used together as a 1 : 3 mixture. According to the manufacturers the ash content of these activated charcoals is of the order of 10%, the main ash constituents being Si, Fe and Al, with smaller amounts of other elements. The

authors' analyses showed a total nitrogen content of approximately 0·5 %. The animal charcoal was reduced before use to the form of granules 0·5–3 mm. in diameter. Animal charcoal is commonly stated to consist chiefly of inorganic phosphates and to include only about 10 % of carbon. The charcoals were mixed with the dry sand prior to filling the culture vessels.

Mineral nutrients were supplied in the form of a Rothamsted solution (Thornton, 1929) or of Hiltner's solution (Virtanen & von Hausen, 1935), both being free of combined nitrogen. Minor elements were supplied as the A–Z solution (Templeman, 1941)*, 1 ml. of this being added per litre of nutrient solution. An amount of nutrient solution calculated to bring the rooting medium to 80 % of its moisture-holding capacity was initially added to the plant containers, and the rooting medium was restored to this level every 2–3 days by adding alternately further solution or distilled water. In experiments involving uninoculated legumes, in order to reduce the likelihood of infection of the plants by the nodule organism, the surface of the sand was protected by a layer of dry gravel, water being supplied through glass tubes penetrating the gravel layer.

Maple field pea (*Pisum arvense* L.) was used, seeds of selected weight being surface-sterilized and subsequently inoculated with an effective pea-nodule organism (HX strain, originally received from Prof. A. I. Virtanen) prior to sowing, unless uninoculated plants were required. As detector plant for excretion of fixed nitrogen, barley (Chevalier) was intersown in equal number with the peas. Control pots with barley alone were included, with twice as many barley plants per pot as in the mixed cultures.

The plant cultures were grown on a well-exposed outdoor site, protected during wet weather by screens of glass substitute supported by a light wooden framework. The experiments were continued until pods were present on the peas, and the position of individual pots was frequently changed. Dry weights were obtained by heating the plant material at 95° C. and total nitrogen by the Kjeldahl process.

Results

The data for peas will be presented first, those relating to the barley plants which in most experiments were intersown with the peas being considered at the end of this section.

The results of the only experiment made to investigate the effect of wood charcoal on the growth of inoculated peas are presented in Table 1. The inclusion of only a single pot with sand alone as the rooting medium is a weakness in the experiment, but the conclusion that the presence of charcoal in the other pots led generally to an increase in dry weight and nitrogen fixation† is supported by the fact that the figures

* 35 mg./l. of molybdenum trioxide was added to the usual constituents of the A–Z solution.

† An estimate of the amount of nitrogen fixed in the experiments included in this paper may be obtained by deducting, from the figures for nitrogen content of the pea plants, the original seed nitrogen (10 mg./seed). This may slightly over-estimate the fixation, since a little nitrogen may have been gained from the sand.

TABLE 1. *Effect of wood and activated charcoal on growth of inoculated peas, growth period 15 June–28 September 1946**

Charcoal added	Dry wt. of plants (g.)	N content of plants (mg.)	Dry wt. of nodules (g.)	No. of nodules
None, sand alone	17·55	654	0·54	5700
2 % wood charcoal	24·07	787	0·61	7140
4 % wood charcoal	25·52	903	0·57	5050
6 % wood charcoal	18·92	676	0·38	3890
8 % wood charcoal	29·10	1031	0·47	4830
2 % activated charcoal	32·66	1150	0·68	4640
4 % activated charcoal	30·24	1067	0·54	3120
6 % activated charcoal	27·84	993	0·46	1680
8 % activated charcoal	21·72	773	0·30	810

* Six peas per unglazed pot containing an equal volume (6·8–8·5 kg.) of rooting medium (coarse sand with or without charcoal). Rothamsted nutrient solution. Data are per pot, with one pot at each treatment. For data for barley intersown with peas see Table 5.

for the latter pots well exceed those for peas grown in sand alone in many previous experiments at the same season.

The first data for activated charcoal are also included in Table 1. A marked increase in dry weight and nitrogen content is shown with the lower proportions of charcoal, the stronger growth (which in this and later experiments was particularly in respect of top growth) being obvious after 4 weeks' growth. Higher proportions of charcoal were less favourable. A marked reduction in the number of nodules accompanied the presence of the charcoal, without any corresponding reduction in nodule dry weight, i.e. the nodules, as confirmed by direct observation, though fewer, were larger and were aggregated to a greater extent near the tap root.

TABLE 2. *Effect of activated charcoal on growth of inoculated peas, growth period 9 July–13 September 1947**

Charcoal added		Dry wt. of plants (g.)	N content of plants (mg.)	Dry wt. of nodules (g.)	No. of nodules
None, sand alone		6·17	214	0·19	970
		5·43	177	0·26	1090
		5·62	220	0·23	870
	Mean	5·74	204	0·23	977
1·5 % sample A		8·72	317	0·31	1020
2·0 % sample A		8·19	280	0·25	450
0·5 % sample B		10·35	415	0·29	350
1·0 % sample B		8·64	345	0·26	220
1·5 % sample B		9·54	364	0·26	155
0·5 % sample C		8·17	313	0·23	410
1·0 % sample C		9·29	376	0·32	320
1·5 % sample C		8·37	329	0·26	220
0·5 % sample D		8·29	298	0·28	830
1·0 % sample D		9·77	343	0·34	880

* Six peas per unglazed pot containing 7 kg. coarse sand with or without charcoal. Rothamsted nutrient solution. Data are per pot. For data of barley intersown with peas see Table 5.

Table 2 shows data of an experiment in which four different samples of activated charcoal were used, dry weight being 40–80% greater than in sand alone, while the increase in nitrogen fixation ranged up to 150%. The reduction in the number (but not in the dry weight) of the nodules in the presence of charcoal is again clearly shown except with sample D, which consisted of powdered charcoal alone.

TABLE 3. *Effect of activated charcoal on growth of inoculated and non-inoculated peas, growth period 16 May–2 August 1948**

	Inoculated		Non-inoculated	
Rooting medium	No. of replicates	Mean dry wt. of plants (g.)	No. of replicates	Mean dry wt. of plants (g.)
Sand alone	8	17·91	6	24·25
Sand + 1% charcoal	8	22·40	7	29·61

The *t* values (Fisher, 1944) for the differences between the means shown above are 6·79 (inoculated series) and 4·40 (non-inoculated series). These values exceed those required for significance at the level $P = 0{\cdot}01$.

* Six peas per unglazed pot containing 7·2 kg. coarse sand with or without charcoal. Rothamsted nutrient solution. The non-inoculated pots received a total of 1500 mg. nitrogen as NH_4NO_3 per pot. Data are per pot.

Table 3 summarizes the results for a further experiment with activated charcoal which included both inoculated and uninoculated plants, the latter being supplied with an ample amount of inorganic combined nitrogen. The data show that the charcoal benefited both types of plant to a very similar extent, and although the effect of the charcoal was smaller than in previous experiments the increases in dry weight are shown to have full statistical significance.

In other experiments with activated charcoal, which will not be reported in detail, indications were obtained that the powdered charcoal was more beneficial to inoculated peas than the granular form, and that the effect of the charcoal tended to be greater in the coarse sand than in the fine sand.

Table 4 indicates that animal charcoal exerted an unfavourable effect on the growth of peas, particularly in Exp. I which was started earlier in the growing season, the peas being very stunted and displaying an intense red pigmentation in the leaves. No further analysis of these effects of animal charcoal has been undertaken.

Data for the barley plants intersown with the peas in most of the above experiments are given in Table 5. Comparing for each rooting medium the dry weight and nitrogen content of barley grown in the same pots as peas with those of barley grown alone, it will be seen that in no case in section A is there evidence of any benefit to the barley by association with peas, despite the enhanced fixation of nitrogen by the latter in the presence of charcoal; this was borne out by the appearance of the plants. In section B, both in sand alone and in sand with activated charcoal, the barley grown with peas shows somewhat higher dry weights and nitrogen content than the control plants. In considering this result, which incidentally is clearly independent of the

TABLE 4. *Effect of animal charcoal on the growth of inoculated peas**

Charcoal added	Dry wt. of plants (g.)		N content of plants (mg.)		Dry wt. of nodules (g.)		No. of nodules	
	I†	II†	I	II	I	II	I	II
None, sand alone	25·37	7·67	872	314	0·34	0·52	3210	3840
	20·54	9·43	763	392	0·32	0·50	—	3760
	22·59	9·63	767	399	0·30	0·47	2540	3850
2 %	—	7·66	—	306	—	0·37	—	3240
3 %	—	9·27	—	377	—	0·37	—	2540
4 %	—	5·52	—	222	—	0·29	—	2360
	—	5·46	—	216	—	0·26	—	1700
6 %	4·15	—	141	—	0·13	—	1020	—
	3·77	—	133	—	0·17	—	2130	—

* Exp. I, 2 May–6 August 1946, eight peas per glazed jar containing 10·3 kg. rooting medium with Hiltner's solution. Exp. II, 12 July–14 September 1947, six peas per unglazed pot containing 8·6 kg. rooting medium with Rothamsted solution. Fine sand was used in both experiments. Data are per pot. For data of barley intersown with peas in Exp. I see Table 5.

† Refer to Exps. I and II.

TABLE 5. *Effect on barley of association with nodulated peas in the presence of charcoals*

	Rooting medium	Type of culture	Dry wt. of barley (g.)†	N content of barley (mg.)†
A*	Sand alone	Barley alone	1·96	22·4
		Mixed	1·15	16·7
	Sand plus 2–8 % wood charcoal	Barley alone	2·18	25·3
		Mixed	1·83	26·4
	Sand plus 2–8 % activated charcoal	Barley alone	1·20	11·5
		Mixed	0·90	10·6
B*	Sand alone	Barley alone	2·68	47·2
		Mixed	3·46	65·5
	Sand plus activated charcoal	Barley alone	2·99	40·1
		Mixed	3·15	52·1
C*	Sand alone	Barley alone	2·15	23·8
		Mixed	2·23	23·7
	Sand plus 6 % animal charcoal	Barley alone	1·82	31·3
		Mixed	3·91	55·3

* For pea data and other details corresponding to sections A, B and C respectively see Tables 1, 2 and 4.

† All data are per pot (six plants per pot in sections A and B, eight plants per pot in section C) and in most cases are means of several pots. As explained in the text, control pots with barley alone actually contained twice as many plants as just indicated, the dry weights and nitrogen contents shown for these pots in the above table being half the values actually obtained. Original nitrogen content per barley grain = 0·9 mg.

presence of the charcoal, it must be noted that the initial available nitrogen content of the sand was greater than in other experiments as shown by the better growth of the control barley in the sand. The detection of relatively slight excretion in the presence of appreciable amounts of initial available nitrogen in the sand is a difficult

task, because, although the density of planting was the same in mixed cultures as in pots with barley alone, there is no guarantee that each of *n* barley plants growing with *n* peas will not take up more of the initial sand nitrogen than each of *2n* barley plants growing alone. In view of this it is preferable that the data in section B should not be regarded as clearly indicative of excretion.

The data in section C of Table 5 show that in the presence of animal charcoal the barley plants associated with peas were definitely superior to those grown alone. Although the peas grown in the medium with animal charcoal were stunted and inhibited, the nodules and roots at the time of harvest were still undecayed. It is possible that excretion did occur from these abnormal pea plants.

The above observations relate to the detection of excretion by examination of associated cereal plants; the alternative procedure of analysis of the rooting medium will be considered in the Discussion.

Comparing data in Table 5 for barley grown alone in sand and in sand plus charcoal, wood charcoal had little direct effect on the barley, but the activated charcoal, though favourable to peas, reduced its growth and nitrogen content, possibly because of adsorption of available sand nitrogen. The animal charcoal, despite its severe inhibition of the growth of peas, had little effect on that of barley.

Discussion

The increases in dry weight and in the amount of nitrogen fixed, which in the above experiments attended the introduction of wood or activated charcoal into sand cultures of inoculated peas, resemble the results obtained by Gukova & Butkevich (1941) for Soya bean with an unstated type of charcoal; but the finding that the beneficial effect extends also to uninoculated peas supplied with combined nitrogen is in disagreement with those authors, although their experiments on this point were not quite conclusive. The mode of action of the charcoal is not necessarily the same with the two types of pea plant, but the similar extent of the benefit (Table 3) rather suggests that it is, i.e. that the charcoal in the case of inoculated plants does not exert its benefit specifically on the nodules and the process of nitrogen fixation, although these may share in some improvement of conditions experienced generally by the root systems.

Examining in more detail the effect of charcoal (particularly the activated type) on inoculated plants, since the increase in nitrogen fixed in the presence of the charcoal greatly exceeds any slight increase in nodule dry weight, it is clear that the nodule tissue is more active in fixation. Thus, referring to Table 1, the ratio Nitrogen fixed/Final nodule dry weight = 594 mg./540 mg. = 1·1 for sand alone, while in the presence of 2% activated charcoal the ratio = 1090 mg./680 mg. = 1·6. The charcoal also led to the development of a very much reduced number of nodules, while the average size of the nodules was increased. Thus in Table 2 the mean dry weight per nodule for plants in sand alone is 0·2 mg., while in the presence of 1·5% of charcoal (sample B) the corresponding figure is 1·7 mg. Although such a change in nodulation has

often been considered advantageous to the legume, it does not here seem to be intimately concerned with the beneficial effect of the charcoal. Thus, in Table 1, the beneficial effect of wood charcoal is not accompanied by any definite reduction in the number of nodules, while with activated charcoal in the same table there is no correspondence between the benefit to the plants and the number of nodules; also there is, as stated already, the probability that the beneficial effect of the charcoal is not exerted specifically via the nodules.

Gukova & Butkevich (1941) considered that the beneficial effect of charcoal observed by them was due to the fine-sand medium being loosened, with consequent improvement in aeration. It seems doubtful that the addition of as small an amount of charcoal as 0·5% (as proved to be beneficial in some of the present experiments) would materially affect aeration; further, the sand used here was mostly of a relatively coarse type.

There is the possibility that the charcoal contains substances of nutrient value to the plants. The question of the availability to the peas of some of the nitrogenous material of the charcoal has been carefully considered. Observations on the growth in sand plus charcoal of uninoculated peas (supplied with nitrogen-free nutrient solution), also the results of Kjeldahl analyses on rooting media including charcoal before and after the growth in them of inoculated peas, indicate that the nitrogen of the charcoal is unavailable to the peas. Again the demonstration of a beneficial effect of charcoal on uninoculated peas supplied with ammonium nitrate makes it generally unlikely that the charcoal acts by virtue of its nitrogen content.

Of other nutrient substances which might be supplied by the charcoal, particular attention has been paid to molybdenum, in view of the evidence obtained by Anderson & Thomas (1946) of its importance for symbiotic nitrogen fixation, especially since the changes in nodulation seemed to bear some resemblance to those noted by the above authors in the presence of molybdenum. As stated, in all the experiments so far described in the present paper, molybdenum was supplied in the culture solution as the trioxide in an amount which was of the same order as that reported as optimal for legumes by Anderson & Thomas, though they used the probably more readily available sodium molybdate. A further experiment was set up comprising sand cultures of inoculated peas with and without molybdenum (here in the form of the molybdate), in order to test whether the molybdenum exerted a benefit corresponding to that of activated charcoal. The plants were severely damaged by gales after 8 weeks' growth, but up to that date no benefit from the molybdenum was shown, whereas the benefit of activated charcoal was already clear. It may also be noted that the effect demonstrated by Anderson & Thomas was confined to nodulated plants not supplied with combined nitrogen and was not shown in plants provided with the latter, whereas in the present experiment we have to explain benefit to both types of plant. On the whole it seems unlikely that molybdenum was concerned in the effects.

The effect of the charcoal may be connected with its adsorptive capacity. The fact that activated charcoal exercised its beneficial effect in much lower proportions than

wood charcoal (Table 1) is in general support of this idea. Prianishnikov & Domontovitch (1926) believed that the charcoal by adsorption prevented the development of unfavourable pH, though Zinzadzé (1932) decided that the buffering action of charcoal was inadequate to explain the effects observed by him. Measurements of pH of rooting media in the present experiments showed that pH in cultures in sand alone was usually close to 6·5, while in the presence of activated charcoal values ranged from 6·8 to 7·3. Since there is some evidence that the yield of peas is reduced if the pH falls below 6·8 (Dillewijn, 1943), it is possible that these relatively small differences of pH had a significant effect on growth and contributed to the beneficial effect of the charcoal.

In addition there is the possibility that the charcoal adsorbs toxic products of metabolism of the root systems or of the micro-organisms present, or adsorbs excess nutrients. The adsorption of toxins was the explanation favoured by Zinzadzé (1932) and by Papadakis (1941). Drainage from the pots in the present experiments was deliberately excluded, the medium being never watered beyond its water-holding capacity, because of the desire to avoid the loss of any excreted products of fixation. Investigations into the existence of root toxins (usually relating to the effect of the roots of one species of plant on those of another) have yielded inconsistent results (Russell, 1937), but it is clear that the present experimental arrangement was conducive to the accumulation and action of any such substances.*

The marked reduction in the number of nodules in the presence of activated charcoal suggests that there must be fewer invasions of the roots by the nodule bacteria, or that a lower proportion of the infection threads forming in the roots result in nodule development. The former seems the more likely, and to explain this one has to assume that the charcoal hinders the growth or spread of the nodule bacteria in the rooting medium, or that the mechanism bringing about entry into the roots is interfered with, e.g. by the charcoal adsorbing the root secretion, which, according to Thornton (1929), induces entry of the bacteria.

In considering whether the enhanced fixation of nitrogen by inoculated peas in the presence of charcoal was attended by excretion of a proportion of the fixed nitrogen, it was shown that in one experiment with wood and activated charcoal the growth of barley provided no evidence of such excretion, while a further experiment with activated charcoal was inconclusive because of the relatively high nitrogen content of the sand, though the excretion was in any case relatively slight. The growth of the barley provided some evidence of excretion from the abnormal pea plants developing in the presence of animal charcoal. With all types of charcoal the possibility must be borne in mind that excretion occurred, but the excreted products were for the most part adsorbed by the charcoal, and the cereals thus excluded from benefit. With this in mind, a considerable number of Kjeldahl analyses of rooting media were carried out, particularly on media containing activated charcoal. The initial presence of relatively

* Gray & Bonner (*Amer. J. Bot.* **35**, 1948, p.52) refer to more recent evidence of excretions by a number of plants having toxic action on other plants of the same or different species.

large amounts of nitrogenous material in the charcoal introduces a large sampling error into analyses of sand-charcoal mixtures and makes it impossible to detect other than gross changes in nitrogen content of the rooting media, of which no evidence was obtained, i.e. the nitrogen content of rooting media after the growth of inoculated peas was essentially the same as at the commencement of the experiment.

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THE EFFECT OF ASSOCIATED SOIL MICROFLORA ON *FUSARIUM UDUM* BUTL., THE FUNGUS CAUSING WILT OF PIGEON-PEA (*CAJANUS CAJAN* (L.) MILLSP.)

By R. S. VASUDEVA, D.Sc. (Lond.), Ph.D. (Lond.), D.I.C.
and T. C. ROY, M.Sc.
Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi

(With 3 Text-figures)

Inoculation with *Fusarium udum* Butl. produced more wilt of pigeon-pea in sterilized than in unsterilized soils at the same pH. From unsterilized soils with low disease incidence, nine fungi, *Bacillus subtilis* and an *Actinomyces* were isolated. The number of isolations of a particular organism varied from month to month during the cropping season of pigeon-pea in Delhi. Interaction of *Fusarium udum* and other organisms isolated was studied. *Aspergillus niger* and *A. terreus* secreted inhibitory substances in potato-dextrose broth: *Bacillus subtilis* inhibited growth on solid medium and also produced a toxic substance in potato-dextrose broth. The nature of the medium employed and period of growth were important factors in the production of the inhibitory principle, which is thermostable. The low incidence of pigeon-pea wilt in unsterilized soils may result from the inhibitory activity of the associated microflora in the soil.

Introductory

The varietal trials of pigeon-pea (*Cajanus cajan* (L.) Millsp.) for wilt made over a number of years in sterilized and unsterilized soils have shown striking differences in the incidence of the disease. This suggests that other micro-organisms in the soil affect the parasitic activity of *Fusarium udum* Butl. Attempts have been made to isolate the species present in the unsterilized soils and to determine their antibiotic activity against *F. udum*.

The extensive literature on the subject of the antagonistic action of soil-inhabiting fungi and bacteria on soil-borne plant pathogens has been reviewed by Sanford (1946), Weindling (1946) and Waksman (1941, 1948). The antagonism exhibited by fungi, particularly species of *Trichoderma*, *Gliocladium*, *Penicillium*, *Helminthosporium*, *Trichothecium* and *Myrothecium verrucaria* against fungi, both soil-borne and otherwise, is well known. Some bacteria have also been found to produce substances antagonistic to soil-borne plant pathogens. Thus Porter (1924, 1932) kept wheat and flax seedlings from infection by *Helminthosporium* and *Fusarium* respectively by the use of antagonistic bacteria. Brown (1933) has recorded that a watermelon disease caused by *Phymatotrichum omnivorum* was reduced by the presence in soil of fungi like *Trichoderma lignorum* and certain bacteria. Species of *Pseudomonas* and *Achromobacter* have been similarly found by Chudiakov (1935) to

cause the lysis of *Fusarium* spp. and other fungi. The spore-forming bacteria *Bacterium simplex* and *Bacillus mesentericus* have also been known to produce substances antagonistic to *Rhizoctonia solani* (Cordon & Haenseler, 1939) and *Helminthosporium sativum* (Christensen & Davies, 1940) respectively.

EXPERIMENTAL

Influence of wilt in sterilized and unsterilized soils

Inoculation experiments were made in 12 in. earthen pots cleaned with 5% lysol solution and then in running water. The pots were either filled with autoclaved soil or unsterilized soil. The soil was inoculated by adding 400 g. of *Fusarium udum* grown on soil maize medium* in 500 ml. Erlenmeyer flasks. Plants of pigeon-pea varieties I.P. 69 and NE(*a*) known to be highly susceptible to wilt were used. Seeds sown in sterilized soil were surface sterilized with 0·1% mercuric chloride for 2 min. Four sets of treatment listed below were maintained with both the varieties:

(*a*) Plants grown in unsterilized soil with inoculum.
(*b*) Plants grown in sterilized soil with inoculum.
(*c*) Plants grown in unsterilized soil without inoculum.
(*d*) Plants grown in sterilized soil without inoculum.

Each treatment consisted of six pots with five plants in each, and all the pots were maintained under the same conditions of light, temperature and irrigation. The initial pH of the soil was 8·2 and remained unaltered throughout the experiment. Observations on the incidence of wilt were made at regular intervals, and the greater incidence in sterilized soil is shown in Table 1 which summarizes the results of a typical experiment. The controls in series (*c*) and (*d*) remained healthy.

TABLE 1. *Incidence of wilt in sterilized and unsterilized soil*

	Variety I.P. 69				Variety NE(*a*)			
	Sterilized		Unsterilized		Sterilized		Unsterilized	
Dates of observation	Plants infected	Infection (%)	Plants infected	Infection (%)	Plants infected	Infection (%)	Plants infected	Infection (%)
20. x. 47	1	3·3	0	0	7	23·3	0	0
20. xi. 47	7	23·3	2	6·6	16	53·3	2	6·6
20. xii. 47	10	33·3	3	10·0	19	63·3	4	13·3
20. i. 48	13	43·3	4	13·3	22	73·3	6	20·0
20. ii. 48	21	70·0	13	43·3	22	73·3	9	30·0

Total plants inoculated in each series = 30.

Isolations were made from the unsterilized soil in such pots on glucose-peptone agar, potato-dextrose agar and nutrient agar and the following organisms were obtained: *Rhizopus nigricans* Ehrenb., *Mucor* sp., *Cunninghamella elegans* Lendner,

* 190 g. of finely sieved soil, 10 g. of ground maize, 70 ml. of water.

Fusarium udum Butler, *Aspergillus niger* van Tiegh., *A. terreus* Thom., *A. amstelodami* (Mangin) Thom. & Church, *Rhizoctonia bataticola* (Taub.) Butler, *Alternaria* sp., *Actinomyces* sp., bacteria (A and B).

The prevalence of different organisms was determined throughout the growth of the crop every month by percentage colonization of wheat straw buried in unsterilized pot soils (Sadasivan, 1939). *Aspergillus* sp., *Rhizoctonia bataticola*, *Alternaria* sp. and bacteria predominated during October, but were much reduced from the end of November. In December, soon after a few light showers, the phycomycetous fungi and *Rhizoctonia bataticola* were more abundant. Fig. 1 shows the fluctuation of micro-organisms at different periods.

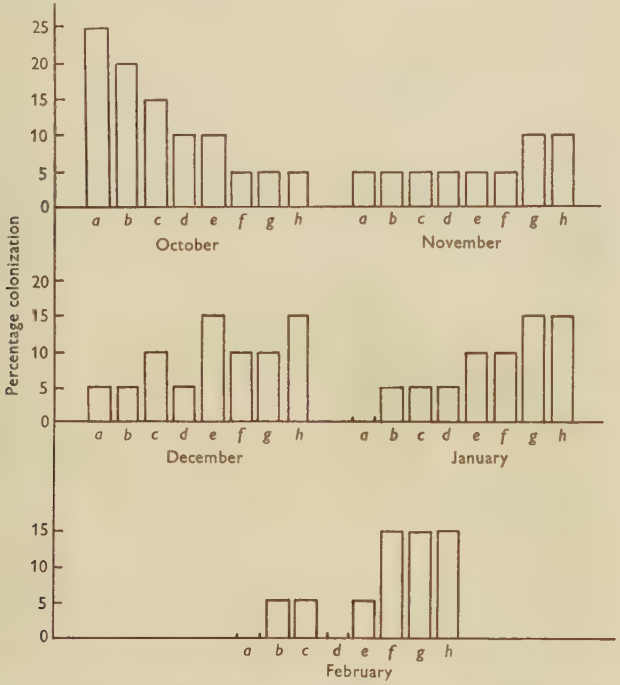

Fig. 1. Showing development of fungi from straws buried in the soils during different months. *a*, *Aspergillus terreus*; *b*, bacteria; *c*, *Aspergillus niger*; *d*, *Alternaria* sp.; *e*, *Rhizoctonia bataticola*; *f*, *Mucor* sp.; *g*, *Rhizopus nigricans*; *h*, *Cunninghamella elegans*.

Interaction of fungi isolated and Fusarium udum

On potato-dextrose agar. Interaction of each of the fungi isolated from the soil with *F. udum* was tested on potato-dextrose agar (pH 6·5) and Richards's agar (pH 5·0) in Petri dishes. The wilt organism and one of the isolates were inoculated 1½ in. apart, (*a*) simultaneously and (*b*) with a 24 hr. start for *F. udum*. The plates were incubated at 30° C. Neither lysis of the hyphae of *F. udum* by any one of the

isolates, nor inhibitory zones between interacting fungi were observed. *Rhizoctonia bataticola*, *Cunninghamella elegans* and *Rhizopus nigricans* grew much faster, so that when the *Fusarium* colony was only 2 cm. in diameter each of them overtook the growth of the wilt organism. *Rhizoctonia bataticola* arrested further growth of *Fusarium udum* after 7 days at a pH below 6·5 and grew completely around the *Fusarium* colony, whereas *Aspergillus niger*, *A. terreus*, *A. amstelodami*, *Alternaria* sp. and *Actinomyces* sp. were compatible with it, which was evident from the intermingling of the colonies.

Interaction of the fungi was also studied on potato-dextrose agar slopes in Petri plates having a median line of thin medium as used by Vasudeva & Sikka (1941). No interaction of hyphae of any of the groups of fungi tested was observed.

Production of inhibitory substance in fluid media. Tests were made to determine whether any of the fungi isolated produced a substance which might inhibit or stimulate the growth of *Fusarium udum*. Each of the isolates was grown in 250 ml. Erlenmeyer flasks on 50 ml. of potato-dextrose solution (Slagg & Fellows, 1947) at 29° C. for 10 days and the culture then filtered through Whatman's filter-paper no. 42. Ten ml. of the filtrate from each culture was autoclaved at 15 lb. pressure for 20 min. One ml. of each autoclaved filtrate was added to 10 ml. of potato-dextrose agar, thoroughly mixed and poured in Petri plates. The plates were inoculated with *F. udum* in triplicate and incubated at 30° C. The linear growth of the fungus was recorded. The results are set out in Table 2.

TABLE 2. *Effect of autoclaved fungal culture liquids on growth of* Fusarium udum

Culture liquid of	Diam. of colonies (mm.) (3 days)	Compared with growth of control (%)
(1) *Aspergillus terreus*	55	73·4
(2) *A. amstelodami*	75	100
(3) *A. niger*	45	60
(4) *Alternaria* sp.	67	89·4
(5) *Cunninghamella elegans*	52*	115·5
(6) *Fusarium udum*	50*	111·1
(7) *Rhizopus nigricans*	70	93·4
(8) *Rhizoctonia bataticola*	75	100
(9) Control	75	100

* Figures indicate 2 days' growth, as on third day the fungus overgrew the plates and hence no reading was possible. Growth of the control after 2 days was 45 mm.

In another experiment unheated fungal filtrates of the same age were passed through L_3 Chamberland candles. Each filtrate was separately incorporated in potato-dextrose agar and tested for the growth of *F. udum* as above (Table 3).

It is clear from the data set out in Tables 2 and 3 that *Aspergillus niger* and *A. terreus* produce inhibitory substances which are thermostable and pass unaffected through L_3 Chamberland candles, whereas the filtrates from *Cunninghamella elegans* and *Fusarium udum* cultures appear to stimulate the growth of *F. udum*.

TABLE 3. *Effect of filtrates passed through* L_3 *candle on growth of* Fusarium udum

Filtrate of the fungus	Diam. of colonies (mm.)	Compared with growth of control (%)
(1) *Aspergillus terreus*	41	58·6
(2) *A. amstelodami*	70	100
(3) *A. niger*	38	54·3
(4) *Alternaria* sp.	68	97·2
(5) *Cunninghamella elegans*	75	107·1
(6) *Fusarium udum*	75	107·1
(7) *Rhizopus nigricans*	68	98·6
(8) *Rhizoctonia bataticola*	70	100
(9) Control	70	100

Interaction of bacteria and Fusarium udum

The interaction of the bacteria isolated from the soil was similarly tested by growing the organisms beside *F. udum* on potato-dextrose agar. The isolates A and B induced inhibitory zones on the third day. The maximum zone was 8 mm. with isolate A and 6 mm. with B. *F. udum* even with 48 hr. start was inhibited to the same extent as when planted simultaneously. No electric potential difference of the two colonies showing the inhibitory zone was observed either on potato-dextrose agar or on Richards's agar with an adjusted pH of 6·0.

pH *of the medium and inhibitory zone.* A set of media was prepared with different percentages of malic acid and sodium bicarbonate to the glucose-peptone agar medium (Vasudeva, 1930), giving a range of pH from 2·8 to 9·4. The two test organisms, bacterial isolate A and *F. udum* were inoculated simultaneously in triplicate at two points, 1 in. apart and incubated at 30° C. The widest inhibitory zone developed between pH 6·0 and 8·6. At pH 5·8 and 8·8 there was a tendency towards inhibition, but the zone was not marked. *F. udum* can tolerate a range of pH 4·6–9·0, the optimum for growth being 5·8.

Determination of lytic action. Bacteria A and B on nutrient broth of different ages varying from 2 to 14 days were tested by the ring method on a 3-day-old culture of *F. udum* at 30° C. The sterilized aluminium rings were placed at four different points on the colony of the fungus grown on nutrient agar and filled with the culture liquid of the bacteria under test. No lysis of the hyphae of *F. udum* was noted even after incubation for 7 days.

Similarly, the growth substance of *F. udum* of varying ages was tested against the bacteria. No lysis of the bacterial colony was observed.

Effect of culture liquid of the bacteria on the growth of Fusarium udum. The bacteria under test were grown in 10 ml. nutrient broth and incubated at 30° C. for different periods. One ml. of the filtrate of a particular age was mixed with 10 ml. of potato-dextrose agar and poured in 3 in. Petri dishes. The plates were inoculated with *F. udum*. The controls were maintained by growing *F. udum* on potato-dextrose

agar alone. The inhibitory effect was pronounced with 4-day-old culture liquid, but was not shown when nutrient agar was used.

The germination of spores of *F. udum* was also tested in the boiled and unboiled culture liquid of both the bacterial isolates at 30° C. Germination of the spores in 4-day-old liquid of the bacteria grown on nutrient broth was not adversely affected, but in 10 ml. potato-dextrose agar mixed with 1 ml. of this culture liquid no colony of *F. udum* developed even after 1 week. The culture liquid of both isolates of bacteria grown on potato-dextrose broth adversely affected spore germination. The conidia after 42 hr. incubation produced thicker germ tubes with wavy walls and balloon-like swellings. No further growth of the germ tubes was observed.

Filtered culture liquids of the two bacteria on potato-dextrose solution were treated as shown in Table 4. One ml. from each of the lots was added to 10 ml. of potato-dextrose agar, thoroughly mixed and poured in Petri dishes. These were then inoculated in quintuplicate and incubated at 30° C. The results show that even the heated culture filtrate much reduced the growth of *F. udum*.

TABLE 4. *Effect of heat on the inhibitory principle*

1 ml. of cultural liquid		Average growth of *F. udum* (cm.) 24 hr.	48 hr.	72 hr.
(1) Bacterium A:	(*a*) Unheated	Nil	Nil	Nil
	(*b*) Heated 10 min. at 100° C.	Nil	0·8	1·1*
	(*c*) Boiled to half the volume	Nil	0·9	1·1*
(2) Bacterium B:	(*a*) Unheated	Nil	Nil	Nil
	(*b*) Heated 10 min. at 100° C.	Nil	1·1	1·2*
	(*c*) Boiled to half the volume	Nil	1·2	1·2*
(3) Control		1·2	5·0	7·5

* No further radial growth even after 14 days, but the colony showed slight aerial growth.

Another experiment was made in which 1 ml. lots of the boiled culture liquid of bacterium A were added to a series of 10 ml. of potato-dextrose agar. The pH of these mixtures was adjusted from 2·8 to 9·2 by the addition of N/10-sulphuric acid and N/10-sodium bicarbonate. Fig. 2 shows growth of *F. udum* at different pH values with and without bacterial filtrate. The peak of the growth is normally at pH 5·8 but is 4·6 in the presence of the inhibitor.

Effect of filtration. Four-day-old culture liquid of bacterium A grown on potato-dextrose agar, after passing through filter-paper impregnated with 2 % Kieselguhr, was filtered through L_3 Chamberland candles. To each lot of 10 ml. of potato-dextrose agar different quantities of the filtrate varying from one drop to 2·5 ml. were added. Growth of *F. udum* decreased with the increase in the amount of the filtrate up to 2·5 ml. but not beyond. Heating the filtrate for 15 min. at 100° C. did not destroy the inhibitor. Fig. 3 shows linear growth of *F. udum* on potato-dextrose agar with

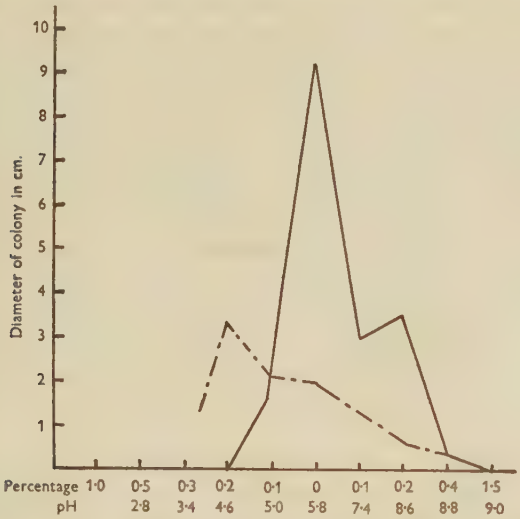

Fig. 2. Growth of *Fusarium udum* on glucose-peptone agar of different pH (3 days' growth) compared with the growth behaviour of the same in presence of filtrate of bacterium A.

———— Without bacterial filtrate. —·—·— With bacterial filtrate.

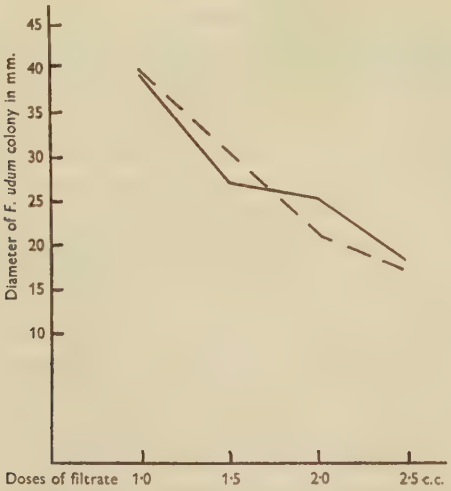

Fig. 3. Growth (3 days) of *Fusarium udum* on potato-dextrose agar with different doses of filtrate heated and unheated.

— — — Heated. ———— Unheated.

varying quantities of heated and unheated filtrates. In control plates without the filtrates *F. udum* showed a growth of 75 mm. in the same period.

Identity of bacteria A and B. Both cultures A and B are aerobic (facultative anaerobes) spore-forming rods, Gram-positive, non-acid-fast, forming no chains. The spores are oval, central to subcentral—sporangia only slightly bulging. The maximum spore diameter of both is 0·95 μ, minimum width 0·65 μ. Maximum spore length: A, 1·70 μ; B, 1·5 μ. The cells are actively motile, grow in nutrient broth with the formation of turbidity and a slight pellicle. Gelatine is liquefied. Culture A tends to give an infundibuliform zone and B a saccate zone. Acid without gas is formed from dextrose and sucrose. Lactose, mannitol and xylose are not attacked. Acetylmethyl carbinol is not formed. The methyl-red test is negative. Nitrate is reduced strongly to nitrite. Indole is not formed. Hydrogen sulphide is not formed. Starch is very strongly hydrolysed. Milk is not attacked in 7 days, but the litmus indicator is reduced. On cooked potato tissue, culture A gives a dry wrinkled yellowish growth, culture B a greyish white wrinkled growth. On nutrient agar the colonies are whitish, moist, smooth, translucent, margin undulating. The agar slants are opaque with abundant growth, white, glistening, soft, slightly adherent. All the cultures were made at 25° C.

The vegetative cells measure about 0·7 × 2–3 μ (mean values).

From the general characters of the cells and the shape and position of the spores, both the cultures A and B appear to fall in the *Bacillus subtilis* group as defined in Bergey's *Manual* (1948). The differences between the two cultures of organisms are small and do not warrant their separation.

From the dimensions of the spores the organisms do not appear to be strains of *B. subtilis* itself, but rather to belong to the section of *B. subtilis* group which has medium-sized spores.

Discussion

The incidence of pigeon-pea wilt is lower in unsterilized soils and the hydrogen-ion concentration is not responsible for variation in disease incidence. Sanford (1926) explained the reduction of activity of *Actinomyces scabies* on the basis of too acidic a soil reaction produced by the associated soil saprophytes. Relative colonization of straw by the soil fungi and bacteria during the growth period of pigeon-pea plants shows considerable variation. The reduction of *Aspergillus terreus*, *A. niger* and *Bacillus subtilis* during the month of November, and their subsequent low level until the end of the cropping season, corresponds to the high incidence of disease from November to February.

Aspergillus niger and *A. terreus*, though compatible with *Fusarium udum* on potato-dextrose agar, like other fungi isolated, secreted inhibitory substance on potato-dextrose broth after 10 days' growth. The principle is thermostable. Broadfoot (1933) recorded similar results for *Ophiobolus graminis* and certain other micro-organisms. The culture filtrates of the fungi *Aspergillus amstelodami*, *Rhizopus nigricans* and *Rhizoctonia bataticola*, showed no inhibitory effect on *Fusarium udum*, but these fungi may exhibit inhibitory action in soil as recorded by Harder (1911), Naegeli (1935) and Waksman & Foster (1937). In our experiments *Bacillus subtilis* appeared to be mainly responsible for inhibition of *Fusarium udum*. The inhibition

was due to a metabolic product of *Bacillus subtilis* and not to any change in the hydrogen-ion concentration of the medium. An inhibitory zone is produced by the bacterium only in the pH range from 6·0 to 8·6.

The production of inhibitory substances in liquid cultures has been shown to depend on the medium used and period of incubation. The effect of medium on the production of inhibitory substances has also been shown by Sanford (1933), Weindling (1934) and Grossbard (1948). It also appears that the toxic principle is partly retained by L_3 Chamberland candles. Sanford & Broadfoot (1931), while studying the effect of certain fungi and bacteria isolated from the soil on the virulence of *Ophiobolus graminis* in pot cultures, found that the cultural fluids of the isolates after passing through Berkefeld filters were less effective in suppressing the virulence of the pathogen. Low incidence of pigeon-pea wilt or suppression of activity of *Fusarium udum* in unsterilized soils can probably be explained because of the inhibitory activity of the associated microflora.

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THE SUBEPIDERMAL FUNGI OF CEREAL GRAINS

I. A SURVEY OF THE WORLD DISTRIBUTION OF FUNGAL MYCELIUM IN WHEAT

By M. B. HYDE

Pest Infestation Laboratory, Slough, Bucks

(With 2 Text-figures)

Examination of samples received in 1947 and 1948 showed that a subepidermal mycelium occurred in normal wheat grains from almost all the wheat-growing areas of the world. The amount of mycelium varied widely: there are indications that the degree of infection is dependent on the atmospheric humidity during the ripening of the grain. No subepidermal mycelium was found in wheat grains from some crops grown under irrigation.

Introduction

One of the chief causes of damage in stored wheat is 'heating', which may be caused either by the respiration of insects or of microbial organisms such as fungi and bacteria. Leach (1944) concluded that the carbon dioxide evolved by grains at a humidity too low to permit germination was not mainly due to the respiration of the wheat grains themselves, but was produced largely by the micro-organisms developing on them.

When the outer pericarp was removed from wheat grains by shaking with coarse carborundum powder, Oxley & Jones (1944) noted that the respiration of the treated grain was appreciably reduced, although the germination of the grain was not affected. They showed that this was mainly due to the respiration of fungi, not on the outside of the grain, as Leach supposed, but beneath the epidermis. Examination of a large number of normal wheat grains showed that while fungal hyphae were not normally present in any quantity on the outside of the grains unless the wheat was obviously mouldy, an extensive mycelium was always found on the inner surface of the epidermis. After a preliminary soaking of the grain in water the epidermis can be quite readily removed, and, on staining with aniline blue, a branched septate mycelium shows up clearly on the inner surface of the epidermis.

Although the presence of internal fungi in cereal seeds has frequently been noted, it was generally in connexion with the examination of diseased grain, and the fungi were assumed to be pathogenic. Some authors, however, refer to the presence of internal fungi in apparently healthy grains, although their location within the grain is not always stated. Bolley (1913), Niethammer (1939), Marcus (1942) and Schwartz-Kraepelin (1947) all report on an internal mycelium occurring in normal wheat and other cereal grains, in a position similar to that described by Oxley

& Jones (1944), but they do not discuss the significance of the fungi. Oxley & Jones found the fungus in the space between the epidermis and the cross-layer (inner pericarp), and further examination has shown that on removal of the epidermis the mycelium is removed with it, only very few hyphae remaining attached to the cross-layer cells. Marcus's account of the mycelium occurring in the space between the pericarp and testa, and growing particularly abundantly in the large cavities at the sides of the crease, suggests a similar position, but it may be that when he says pericarp (*Fruchtschale*) Marcus means epidermis. There is no cavity between the inner (cross-layer) cells of the pericarp and the testa.

The present paper shows the distribution of such fungi among samples of wheat obtained from most of the important wheat-growing areas of the world.

Material and methods

Individuals and research institutions responded most generously to a request for wheat grown in their neighbourhoods during either the 1947–8 or 1948–9 seasons in the Northern Hemisphere or the 1948 season in the Southern Hemisphere. Where possible, samples consisting of five to ten unthreshed ears of each of several varieties from the current harvest were sent in water-tight containers. The ears were threshed by hand and their moisture contents determined by the vacuum desiccator method of Oxley (1948). The samples were stored in 1–4 oz. screw-cap bottles, each with a small bag containing silica gel to maintain low humidity. If practicable the samples had, before dispatch, been air-dried without artificial heat to a moisture content of less than 10% and most samples from distant countries were sent by air-mail, so there was little likelihood of extensive fungus growth *en route*.

A quantitative method of assessing the amount of mycelium present was developed. Examination of a number of wheat grains had shown that the subepidermal fungus tends to occur in a number of clearly defined areas, shown shaded in Fig. 1, which represents diagrammatically the shape of epidermis stripped whole from grains. Each of these areas was allotted a number which represents the proportion, in tenths of the total area, covered with fungus. For example, 1, means $\frac{1}{10}$th covered, 6, means $\frac{6}{10}$ths covered. The density of growth could also be estimated roughly and was expressed arbitrarily by a figure from 1 to 5. The amount of fungus present was estimated relatively by taking the product of the density and distribution numbers. This product was called the fungus 'score'. It was found that samples of eighteen grains gave mean results which were statistically significant for comparing different varieties, locations, etc.

Where a large number of samples was received from one sampling point, a few, generally about six, representative varieties were examined. In some instances only one or two samples from a particular place were available, but the results from these are included, although giving less accurate mean scores for the areas concerned. Examination of a larger number of samples over a prolonged period, although desirable, was not practicable.

The results as given represent the mean values for individual sampling points, and are not averages for a particular area or country.

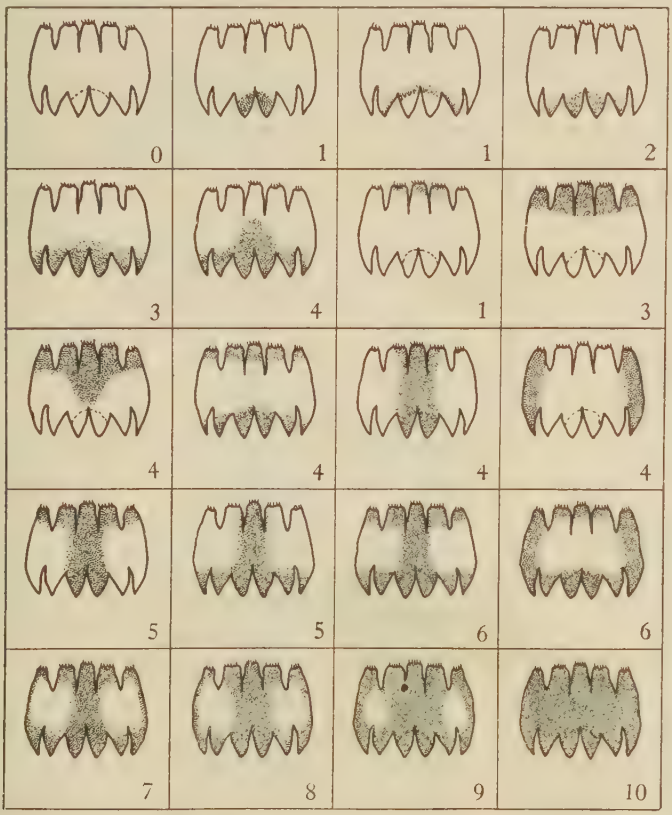

Fig. 1. Diagrammatic representation of distribution of fungal mycelium within the epidermis. (The shading indicates the presence of fungal hyphae.)

Results* and discussion

The fungal scores obtained for each sampling point are summarized in Table 1. The amount of mycelium present, as indicated by fungal score, varied considerably, but tended to be more or less constant for each locality. Such variation as occurred was presumably due to varietal differences which will be discussed later.

Non-climatic factors affecting amount of fungus present

It is not useful to make exact comparisons between the fungal scores from different localities, as there are a number of variable factors, such as climate, growth conditions,

* Complete numerical results are available at the Pest Infestation Laboratory.

TABLE 1. *Amount of subepidermal mycelium in wheats from different parts of the world*

Locality	No. of samples	Mean score
Tournai, Belgium	1	28·6
Orgerons, France	3	20·6 ± 2·2
Gembloux, Belgium	6	20·2 ± 1·3
Angus, Scotland	1	19·4
Moray, Scotland	1	18·9
Meigle, Scotland	1	18·7
Dundee, Scotland	1	18·5
Ross, Scotland	1	18·3
Louvain, Belgium	2	17·5 ± 1·0
Roxburgh, Scotland	1	17·3
Market Weighton, York	2	16·1 ± 2·2
Lough, Eire	2	15·4 ± 2·2
Nagradowice, Poland	1	15·3
Andrezen, France	2	15·2 ± 0·05
Waterford, Eire	1	14·5
Cambridge (England)	4	14·2 ± 1·3
Newton Abbot, Devon	4	13·5 ± 1·5
Warsaw, Poland	2	13·4 ± 0·8
Cork, Eire	1	12·7
Limerick, Eire	1	12·4
Detmold, Germany	7	12·2 ± 0·4
Krefeld, Germany	4	11·3 ± 0·4
Oslo, Norway	3	11·2 ± 0·8
Eszterhaza, Hungary	1	10·9
Rome, Italy	6	10·8 ± 1·2
Celbowo, Poland	1	10·8
Komorow, Poland	1	10·1
Bodö, Norway	2	9·8 ± 6·2
Turin, Italy	6	8·6 ± 1·6
Mochow, Poland	1	8·4
Prague, Czechoslovakia	7	8·3 ± 1·9
Bankut, Hungary	3	6·8 ± 0·3
Elvas, Portugal	6	6·6 ± 1·1
Zamarte, Poland	1	6·3
Bydgoszck, Poland	7	5·7 ± 0·4
Zaragoza, Spain	8	4·7 ± 0·4
Kompolt, Hungary	1	3·2
Hatvan, Hungary	1	2·9
Oslo, Norway	5	1·2 ± 0·2
Wooster, Ohio	2	18·7 ± 1·5
Transcona, Winnipeg	1	17·5
Urbana, Illinois	2	15·3 ± 0·6
Manhattan, Kansas	7	15·2 ± 2·5
Saanichton, B.C.	4	12·7 ± 1·0
Fort Hays, Kansas	4	9·7 ± 1·7
Petersfield, Winnipeg	1	8·8
Union Point, Winnipeg	2	7·0 ± 1·4
St Paul, Minnesota	1	4·6
Fargo, N. Dakota	3	2·4 ± 0·1
Brookings, S. Dakota	2	0·8 ± 0·7
Chapingo, N. Mexico	3	5·7 ± 1·5
Ciudad Obregon Sonora, S. Mexico	3	0·23 ± 0·4
Curitiba, Brazil	6	25·9 ± 2·3
Rio Grande do Sul, Brazil	2	13·0 ± 1·8
Castelar, Argentina	3	10·7 ± 2·7
Belo Horizonte, Brazil	5	5·0 ± 1·1
Los Angeles, Chile	1	4·2
Hospital, Chile	2	2·9 ± 2·0
Paine, Chile	7	2·5 ± 0·8
Guatrache, La Pampa, Argentina	3	2·4 ± 0·6
Potchefstroom, Transvaal	6	10·6 ± 1·3
Gemeiza, Egypt	1	6·8
Giza, Egypt	3	6·0 ± 3·2
Kano, Nigeria	2	2·9 ± 0·6
Arusha, Tanganyika	5	2·8 ± 1·0
Vaalhartz, Transvaal	6	2·3 ± 0·8
Shandawil, Egypt	2	1·6 ± 1·0
Kambowa, N. Rhodesia	1	0
Nanking, China	6	16·6 ± 1·8
Hangchow, China	4	14·8 ± 3·0
Kunming, China	3	10·4 ± 3·6
Taiwan, China	4	7·9 ± 1·8
Ankara, Turkey	6	7·7 ± 2·2
Peiping, China	3	6·9 ± 1·5
Shensi, China	2	6·2 ± 1·0
The Lebanon	2	3·5 ± 0·9
New Delhi, India	1	3·2
Baghdad, Iraq	4	2·1 ± 0·9
Ta-li Hsien, China	1	2·0
Lincoln, Canterbury, New Zealand	6	15·4 ± 1·8
Curlewis, N.S.W.	2	15·2 ± 0·5
Tichborne, N.S.W.	4	10·7 ± 1·8
Gatton, Queensland	1	5·4
Adelaide, S. Australia	3	5·0 ± 1·1
Hermitage, Queensland	2	4·6 ± 1·6
Roma, Queensland	1	3·9
Werribee, Victoria	11	3·2 ± 0·5
Wongan Hills, W. Australia	1	3·2
Merredin, W. Australia	1	0·9

treatment at harvest and after, variety of wheat, etc., which are different for the different regions. Within one sampling point, however, where these factors, except for wheat variety, are constant, the comparatively low standard errors of the mean scores indicate that variation in amount of fungus is not closely related to variety, but is determined more by differences due to climatic or other regional factors. Although, in general, wheat varieties grown in one particular country were not widely grown elsewhere, some varieties were received from more than one region, and the results obtained from them confirm this dominant effect of environmental conditions over variety (see Table 2).

TABLE 2. *Effect of environmental conditions on the fungus score of the same variety grown in different localities*

Variety	Locality	Fungal score
Atlé	Waterford, Eire	14·5
	Newton Abbot, England (S.W.)	13·2
	Limerick, Eire	12·4
	Cambridge, England (E.)	11·5
Bencubbin	Sydney, New South Wales	11·6
	Adelaide, S. Australia	7·0
	Wongan Hills, W. Australia	3·2
	Werribee, Victoria	1·4
	Werribee, Victoria	1·1
	Merredin, W. Australia	0·9
Fylgia	Gembloux, Belgium	18·9
	Market Weighton, England (N.E.)	13·9
	Cork, Eire	12·7
	Andrezen, France	10·7
	Paris, France	10·1
	Paris, France	7·3
Kanred	Fort Hays, Kansas	10·9
	Potchefstroom, Transvaal (W.)	8·4
	Paine, Chile	0·3
Mentana	Turin, Italy	13·3
	Ankara, Turkey	10·1
	Saragossa, Spain	6·3
	Chapingo, Mexico	3·9
	Hospital, Chile	0·8
Punjab	Potchefstroom, Transvaal (W.)	10·1
	Baghdad, Iraq	2·2
	Pretoria, Transvaal (E.)	0·3

Variety has some effect on amount of fungus. For instance, the scores for Mentana wheat, a variety occurring in several localities, tend, except in Mexico and Chile, where all scores were particularly low, to be among the highest in those regions where it occurs (Turkey, Italy and Spain), whereas the scores for Fylgia, also grown in a number of districts, are usually about or slightly below the average for each region.

The mean score for durum wheats was about half that of other wheats. This is

probably due to durum wheats being grown more commonly in the drier countries, where all varieties and types had low fungal scores.

Results from a representative proportion of all the varieties examined showed that there was an interesting inverse correlation, which was statistically significant ($r = -0{\cdot}33$, $P < 0{\cdot}02$), between the fungal score of each sample and the time required to strip the epidermis from the eighteen grains examined, i.e. its adherence. Whether the higher score is produced because the hyphae can spread more rapidly in the greater space available beneath the looser epidermis, or whether the activity of a rapidly developing mycelium causes a loosening from the cross-layer cells is not known. The type of growth found suggests that the former explanation is more likely.

Climatic factors affecting amount of fungus present

There was a tendency towards higher fungal scores in localities where higher relative humidities prevail (i.e. in temperate regions and near the coast in warmer countries), and lower scores in territories where the atmosphere is relatively dry (e.g. the Middle East, Mexico, and the centre of such land masses as U.S.A., China, Africa, etc.). This tendency shows clearly in the fall in fungal score from north-west Europe to the Middle East.

In most places the harvest conditions had been good—the 1947 season in north-west Europe had been particularly dry—but in some areas, e.g. Scotland (1948), British Columbia (1948), Ohio, and Kansas, unusually wet conditions were reported and the stooks stood in the fields for some weeks after cutting. This may have resulted in the fungal scores for these regions being somewhat higher than they might normally be. Conversely, exceptionally dry conditions were reported from Eire, Italy (north), Hungary, and north and south Dakota, and the values for these areas may therefore be lower than average.

Collaborators were asked to indicate the weather conditions prevailing locally before harvest, and detailed meteorological data were available for twenty-seven of the eighty-nine sampling points. Such data for relative humidity (at 09.00 hr.) and temperature (daily mean) as have been provided are given in Table 3, together with the fungal scores, and the values for these relative humidities and fungal scores are plotted in Fig. 2. In this somewhat limited number of examples there is a clear correlation between the amount of internal fungus and the humidity of the atmosphere in the few weeks before harvest.* The correlation coefficient (r) is 0·8287 ($P < 0{\cdot}001$).

The correlation coefficient of the figures for fungus score and mean temperature for the fortnight before harvest is $-0{\cdot}5863$ ($P < 0{\cdot}01$), indicating that there is a statistically significant inverse correlation between amount of internal fungus and temperature. It might be expected that fungal growth would be increased by high

* Investigations in 1948 on Bersée wheat at Slough and on a number of varieties at Heston had shown that it is at this comparatively late stage, namely, after the ears have turned yellow and the grains begun to dry out, that the subepidermal fungi are first seen.

TABLE 3. *Atmospheric humidity and temperature in relation to amount of fungus*

Sampling point	Fungal score	Relative humidity (%)	Mean temp. (° C.)
Curitiba, Brazil	25·9	84	17·5
Gembloux, Belgium	20·2	85	20·6
Market Weighton, England	16·1	82	15·0
Lincoln, New Zealand	15·4	71	14·4
Urbana, Illinois	15·3	76	—
Curlewis, New South Wales	15·2	62	18·5
Cambridge, England	14·2	79	16·5
Newton Abbot, England	13·5	82	16·0
Saanichton, B.C., Canada	12·7	82	15·8
Slough, England	12·7	71	15·4
Rome, Italy	10·8	63	22·4
Tichborne, New South Wales	10·7	78	17·0
Winnipeg, Canada	10·1	77	19·0
Taiwan, China	7·9	69	16·5
Peiping, China	6·9	53	25·5
Gemeiza, Egypt	6·8	70	22·1
Elvas, Portugal	6·6	56	22·3
Shensi, China	6·2	49	23·8
Giza, Egypt	6·0	46	27·0
Adelaide, S. Australia	5·0	50	20·7
St Paul, Minnesota	4·6	52	23·7
Saragossa, Spain	4·7	46	21·6
Paine, Chile	2·5	43	18·6
Fargo, N. Dakota	2·4	57	—
Merredin, W. Australia	2·1	60	18·9
Shandawil, Egypt	1·6	50	24·4
Brookings, S. Dakota	0·8	54	26·9

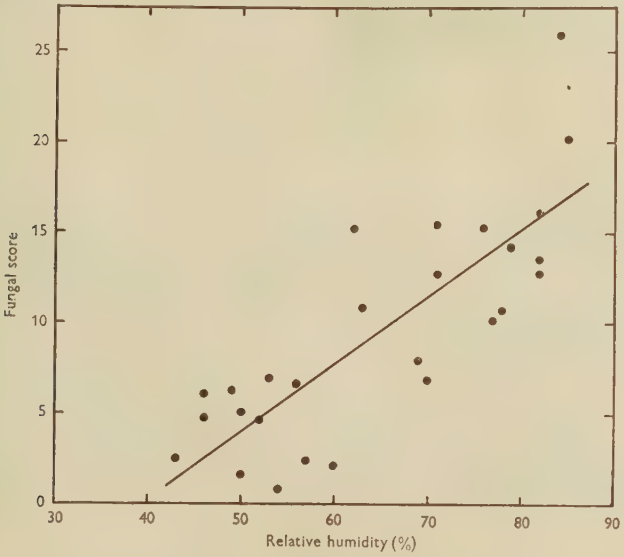

Fig. 2. Relation between atmospheric humidity before harvest and amount of subepidermal fungus.

temperatures, but these are often associated with low humidities, which apparently have a greater effect in retarding it.

No correlation was found between fungal score and moisture content of the grains as determined on arrival. As the moisture content values were on the whole fairly low ($< 14\%$), further growth of fungi during transit was unlikely.

The subepidermal mycelium is not confined to narrow geographical regions, but occurs wherever wheat is grown. Of the 278 samples examined from eighty-nine sampling points, only five varieties from five districts (in Turkey, South Dakota, Mexico, Chile and Northern Rhodesia) had no internal fungus, although, except for Northern Rhodesia (where only one sample was available) fungus was present in other varieties from these localities. Several distinct types of mycelium occur and their identity will be discussed in a later paper.

This survey would not have been possible without the generous co-operation of workers in many parts of the world. Those who so willingly provided samples are too numerous to mention, but in particular thanks are due to Prof. W. F. Geddes, University of Minnesota, who passed on the request to a number of his colleagues, and to Dr T. Verschoyle, Agricultural Department, British Council, and the British Council Scientific Representatives in many countries, who arranged for samples to be sent from local institutions.

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STUDIES ON THE PHYSIOLOGIC SPECIALIZATION OF YELLOW RUST (*PUCCINIA GLUMARUM* (SCHM.) ERIKSS. & HENN.) IN GREAT BRITAIN

By J. G. MANNERS*

Botany School, University of Cambridge

(With Plates 9 and 10 and 3 Text-figures)

During 1945–8, 223 British collections of yellow rust were determined on Gassner & Straib's differential hosts, with the addition of the wheat variety Wilma. Physiologic races 2, 3, 5, 6, 7, 8 and 17 were obtained from wheat, race 46 from barley, race 33 from *Hordeum murinum* and race 28 from *Agropyron repens*. Three new races, 6*x* from wheat, *M* from *H. marinum* and *G* from *Dactylis glomerata* were also isolated. High temperatures increase the susceptibility of some differential hosts to certain wheat races, but decrease that of others. Biotypes of races 2, 6 and 8, differing from the 'type' cultures of those races, were isolated.

Of the commoner wheat races, nos. 6 and 8, occurring on many wheats, were widespread, but races 5 and 7, on fewer varieties, were confined to the north of Britain, and races 2 and 3, on very few varieties, to the south. Certain grasses are partially susceptible to some of the cereal races. The uredospores of race 2 germinate less well than those of races 5 or 8, other wheat races being intermediate. The optimum temperature for spore germination of race *G* is 22·5° C., that of all other races 10–13° C.

Twelve wheat varieties were inoculated in the field with each of the eight wheat races, and some varieties developed field resistance to certain races. The races attacking a variety the most severely in the field inoculations were usually the races isolated from that variety in collections received.

Introduction

Eriksson (1894) divided *Puccinia glumarum* into five formae speciales, attacking different cereals or grasses. Rudorf (1929) showed that American and German collections of yellow rust from wheat probably differed physiologically, but Allison & Isenbeck (1930) were the first to establish the existence of physiologic races in *P. glumarum tritici*. Four races were isolated from wheat in Europe with the aid of ten differential hosts. Wilhelm (1931) isolated five races from various parts of Europe with the aid of ten differential hosts, all different from those employed by Allison & Isenbeck (1930).

Between 1930 and 1939, Gassner & Straib (1932, 1934*a*, *b*) and Straib (1935*a*, 1937*a*, 1939*a*) made a detailed study of the physiologic specialization of *P. glumarum*. The differential series consisted of eleven wheats, namely Michigan Amber, Blé rouge d'Ecosse, Strubes Dickkopf, Webster C.I. 3780, Holzapfels Früh, Vilmorin 23,

* Now at the Department of Botany, University College, Southampton.

Heines Kolben, Carstens Dickkopf V, Spalding's Prolific, Chinese 166 and Rouge prolifique barbu. *Triticum dicoccum* var. *tricoccum*, Fong Tien barley and Heils Franken barley were added by Straib (1935*a*), Estanzuela 75*a* barley by Straib (1937*a*), and Petkuser rye by Straib (1939*a*). Gassner & Straib (1932, 1934*a*, *b*) and Straib (1935*a*, 1937*a*, 1939*a*) described forty-seven races from Europe, Asia and North and South America. Four races were isolated from barley, one from rye, one from *Hordeum murinum* and three from *Agropyron repens*: the others were primarily wheat races. Races 4 and 6 were isolated from English collections in 1932, 1933 and 1934.

Gassner & Straib (1934*b*) concluded that, since certain wheat races infected other cereals and grasses, the concept of formae speciales in the sense of Eriksson (1894) was no longer tenable, and that the species *Puccinia glumarum* should be regarded as consisting of a large number of physiologic races, of which many attacked most strongly one particular cereal or grass. Straib (1937*b*) stated that most physiologic races of yellow rust were more or less restricted geographically. No important changes in the relative importance of the various races had been observed.

Becker (1933) added Kinney and Panzer III to Gassner & Straib's differential hosts, and described five new races from wheat in Europe, one from an English collection. Two races from wheat in North America were described by Bever (1934) who employed Gassner & Straib's differential hosts, with the addition of the wheats Red Russian, Triplet and Black Winter Emmer. These seven races were incorporated in lists compiled by Stakman, Levine, Christiansen & Isenbeck (1935) and Roemer, Fuchs & Isenbeck (1938), but were not accepted by Straib (1935*a*, 1937*a*, 1939*a*), who disregarded them when revising his numbering system. Küderling (1936) isolated two slightly different strains of race 8. This is apparently the first suggestion that certain races of *P. glumarum* include more than one biotype.

Several new races of yellow rust have been described since 1939. Mehta (1940), using Gassner & Straib's differential hosts, isolated four new races in India, lettered *A*, *D*, *E* and *F* by him. Straib (1941) described five new races, no. 48 from barley in Germany, nos. 49, 50 and 51 from wheat in Germany, and no. 52 from wheat in Tanganyika. Becker (1942, 1944), employing Gassner & Straib's differential hosts, described races 53 and 54 from Europe. Fang (1944) reported the occurrence of nine races in China, employing five of Gassner & Straib's wheats and three others as differential hosts. These races cannot readily be compared with those isolated by other workers. However, most, if not all of Fang's races are probably new, since they all attack Chinese 166, a wheat susceptible to only three of Gassner & Straib's races.

The determination of physiologic races

Materials and methods

(i) *The establishment and maintenance of stock cultures*

Except for slight modifications, the methods employed were those described by Gassner & Straib (1932). Collections of yellow rust were obtained from many parts of Great Britain, thanks to the co-operation of the Scottish Plant Pathology Service, the National Agricultural Advisory Service plant pathologists and others, to whom requests for material were circulated in 1945, 1946 and 1947. Collections were also made by the author in the vicinity of Cambridge.

Spores were stored at 2° C. and 40% R.H. These conditions were obtained by placing the spore-bearing leaves in paper packets over 50% sulphuric acid in desiccators inside a refrigerator. Spores maintained their viability under such conditions for at least 4 months, and sometimes for as long as 9 months. Collections from wheat were maintained on Norka and collections from barley on *Hordeum spontaneum*, both of which resist *Erysiphe graminis*, but are very susceptible to yellow rust, even at relatively high temperatures. Cultures from grasses were maintained on the original host species, or on *Triticum dicoccum* var. *tricoccum* or Fong Tien barley, both of which proved to be susceptible to most grass races.

Inoculations for stock cultures were made with a sterile scalpel on the second leaves of seedlings about 14 days old. After inoculation, the plants were incubated for 48 hr. under bell jars standing in a shallow tray of water. In hot weather the bell jars were cooled with a water-spraying device. At the end of the incubation period the plants were transferred to the greenhouse bench and covered with spore-proof 'Cellophane' cases. Pustules appeared 10–12 days after inoculation in summer, 13–18 days in winter. In winter the greenhouse temperature was maintained at 15° C., but in summer this was impossible, and when the average temperature rose above 25° C., as in July 1945, and again in June and August 1947, cultures could not be maintained in the greenhouse, but were regenerated, when cooler weather returned, from stocks stored in the refrigerator.

A single-spore culture was made from one collection of each race, and from a number of other collections which it was desired to examine critically. Too many collections were received to make a single spore culture from each. The dry-needle method (Hanna, 1924) was employed. Under optimum conditions, 5·5% of the spores inoculated caused infections. Sixty-four single-spore cultures were made from forty different collections of yellow rust. No evidence was obtained that more than one race was present in any one collection.

(ii) *The differentiation of physiologic races*

For tests on the differential hosts, the standard conditions laid down by Gassner & Straib (1932), namely adequate, but diffuse daylight, an average temperature of

15° C. and approximately 80% R.H., were adhered to as closely as possible. Experiments were made only in spring, from the middle of March until the end of May, and in autumn, from the middle of September until the end of October, when the light was adequate and temperature not too high.

The differential hosts were grown in a rust-free greenhouse, three plants of each variety in a 4 in. plant-pot being normally employed for each test. Inoculation and incubation were as described above for stock cultures, except that the first leaf, not the second, was inoculated. The plants were inoculated 10–14 days after sowing, when the second leaf was just visible.

The sixteen differential hosts employed by Straib (1939*a*) were used. Seed of all sixteen varieties was obtained from stocks derived from Gassner & Straib's pure lines and maintained at Cambridge. The stock of Webster contained nearly 20% of seed susceptible to all races of yellow rust, this wheat being genetically unstable. Consequently, it was necessary to inoculate six, not three, seedlings of Webster in each test, to obtain a reliable reading. Carstens Dickkopf V, Petkuser rye and Heils Frankengerste contained a very few plants susceptible to all wheat races.

In May 1946 two single spore lines, both assigned to race 6, were differentiated by the wheat variety Wilma, which was resistant to one line (no. 1), but susceptible to the other (no. 23) (see Pl. 9, fig. 1). Accordingly Wilma was added to the differential series. The seed employed was obtained originally from Messrs Garton of Warrington, Lancs, the original breeders.

Tests on the differential hosts were examined 14 and 17 days after inoculation, the highest infection type seen being recorded. The scale of infection employed was that described by Gassner & Straib (1932).

Physiologic races isolated

From 1945 to 1948, 254 collections of yellow rust were examined. 192 collections came from wheat, 23 from barley, 3 from *Agropyron repens*, 2 from *Dactylis glomerata*, 1 from *Hordeum marinum* and 2 from *H. murinum*. Thirteen physiologic races were isolated, and their reactions on the differential hosts are given in Table 1. The eight wheat races and the races from barley, *H. murinum* and *Agropyron repens*, have been characterized by Straib (1937*a*, 1939*a*), and the numbers assigned by him to those races have been adopted by the writer. Races 2, 3, 5, 6, 7, 8 and 17 are wheat races (race 6*x*, differentiated from race 6 by its reaction on Wilma, will be considered later). Race 46 was isolated from barley, race 33 from *Hordeum murinum* and race 28 from *Agropyron repens*. The races isolated from *Hordeum marinum* and *Dactylis glomerata* are new, and have been given the letters *M* and *G* respectively, pending the assignment of numbers to them in Straib's system.

(i) *Races from wheat*

Races 2 and 3 are distinguished from the other wheat races by their ability to attack Holzapfels Früh and Vilmorin 23. They are separable on Rouge prolifique

TABLE 1. *Reactions of physiologic races of* Puccinia glumarum *isolated from British collections*

Usual host ...	Wheat								Barley	*Hordeum murinum*	*Agropyron repens*	*Hordeum marinum*	*Dactylis glomerata*
Physiologic race ...	2	3	5	7	17	6	6*a*	8	46	33	28	*M*	*G*
Michigan Amber	3–4	3–4	4	4	4	4	3–4	4	0+	0–2	0	0	i
Blé rouge d'Ecosse	3–4	3–4	3–4	3–4	3–4	3–4	3–4	3–4	0 0	0 0	0	0 0	i
Strubes Dickkopf	4	4	4	4	4	4	4	4	0 0	0 0	0 0	0 0	i
Webster C.I. 3780	3–4	3–4	3±	0+	3–4	2–4	3–4	0–1	0 0	0 0	0	0	i
Holzapfels Früh	4	4	2–	2–	0–2	0–2	0	0–3	0 0	0 0	0 0	0 0	i
Vilmorin 23	4	4	0–1	0–1	0–1	0	0	0–1	0	0	0 0	0 0	i
Heines Kolben	0(3)	0	0	0	0	0	0	0	0+	0	0	0	i
Carstens Dickkopf V	0–2	0–2	4	4	0–2	0–2	0–1	0–2	0 0	0 0	0 0	0 0	i
Spalding's Prolific	0–2	0–2	2±	2±	4	0–2	0–1	0–2	0 0	0 0	0 0	0 0	i
Chinese 166	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0	0 0	0 0	0	i
Rouge prolifique barbu	1–4	0–2	0–2	0–2	1–2	0–1	0	0–2	0	0 0	0 0	0	i
T. dicoccum var. *tricoccum*	4	4	4	4	4	4	4	4	3–4	4	4	0–1	i
Gerste von Fong Tien	3–	3–	3–	3–	3–	3–	3–	3–	4	3–4	3–	3–	
Heils Dornberger Frankengerste	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0	0	i
Estanzuela 75*a*	i	i	i	i	i	i	i	i	4	0–3	0–1	0–3	i
Petkuser rye	i	i	i	i	i	i	i	i	i	i	i	i	i
Wilma	2–4	1–3	4	4	3–4	4	0–2	4 (1–3)	0 0	0 0	0 0	0 0	i

The symbol i denotes an apparently immune reaction

TABLE 2. *The effect of temperature on the reactions of the differential hosts to the wheat races*

Physiologic race ...	2			3			5			7			17			6			6*a*			8		
Temperature (° C.)	12	15	18	12	15	18	12	15	18	12	15	18	12	15	18	12	15	18	12	15	18	12	15	18
Michigan Amber	4	4	3	3–4	4	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3	4	4	4
Blé rouge d'Ecosse	4	4	4	4	4	3–4	4	4	3–4	4	4	4	4	4	4	4	4	4	4	4	3	4	4	4
Strubes Dickkopf	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Webster C.I. 3780	3–4	3	3	3–4	3–4	2–3	3	3–4	2–3	0	0	0	4	3–4	3–4	2–3	1–2	2±	3–4	3–4	3–4	0	0–2	0–1
Holzapfels Früh	4	4	4	4	4	4	0–1	2–3	3±	0–1	2–3	4	0–1	0–1	2	0	0	2–3	0	0–2	2	0	0–3	3–4
Vilmorin 23	4	4	4	4	4	4	0	0–1	1–2	0	0	1–2	0	0	0–1	0	0	0	0 0	0	0	0	0	1
Heines Kolben	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Carstens Dickkopf V	0	0–1	1–2	0	0–2	1–2	4	4	4	4	4	4	0	0–1	1–2	0	0	1–2	0	0	1–2	0	0	2–3
Spalding's Prolific	0	0	0–2	0	0–1	0–1	0	2–3	2–3	0	1	1–2	4	4	4	0	0–2	1	0	1–2	1	0	0–1	0–2
Chinese 166	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
Rouge prolifique barbu	1–2	2–3	3	0–1	0–2	1–2	0	0	2–3	0	0	2	0–1	1	2–3	0	0	1	0	0–1	1	0	0	2
Wilma	2–3	2–3	4	—	3±	3–4	4	4	4	4	4	4	3–4	3–4	4	4	4	4	0–1	1	1–2	4	4	4

The average temperatures quoted above were calculated from the record sheets of a continuously recording thermohygrograph. The readings at 12° C. were obtained in October 1946, those at 15° C. in September 1947 and May 1948, and those at 18° C. in May 1947.

barbu which is susceptible to race 2, but resistant to race 3. Spalding's Prolific gave a type 2 – reaction to race 2 at Cambridge and not the type 3 – reaction recorded by Straib. This difference is probably not significant, since the reactions of Spalding's Prolific are influenced by slight changes in environmental conditions. Race 4, similar to race 3, but giving an immune reaction on Spalding's Prolific, was not isolated, though single samples of yellow rust sent to Canada from England in 1931 and 1932, and determined by Newton & Johnson (1936), belonged to race 4. Collections made at Cambridge in 1932 and 1933 proved to be race 4 (Gassner & Straib, 1934*a*, *b*), but a sample sent from Cambridge in 1934 belonged to race 6 (Straib, 1935*a*). Race 4 probably has become less common than formerly: Straib (1935*a*) isolated race 4 thirteen times between 1931 and 1933, but only twice between 1934 and 1938.

Races 5 and 7 are characterized by the susceptibility of Carstens Dickkopf V to both of them, and are separated only by the susceptibility of Webster to race 5, and its resistance to race 7. Straib (1939*a*) established a new race, no. 41, as a segregate from race 7. He stated that Webster gave a type 4–3 reaction to race 5, a type 2–3 reaction to race 41 and a type 0–1 reaction to race 7. The reactions of Webster to most races are strongly influenced by environmental conditions (see p. 194) and, in the writer's experience, race 5 gave a type 2–4 reaction on Webster, and race 7 a type 0–2 reaction, under conditions approaching those laid down by Gassner & Straib (1932). No evidence was obtained for the existence of race 41 as a separate entity, and it seems best to regard that race merely as a biotype of race 5 or race 7.

Race 17 is characterized by its ability to attack Spalding's Prolific. The resistant reaction of Rouge prolifique barbu distinguishes it from the otherwise similar race 22. Races 6 and 8, the least virulent of the wheat races, are separable by their reactions on Webster, which is susceptible to race 6, but resistant to race 8, under standard conditions. In the spring of 1946, one single spore line (no. 1), attributed to race 6, gave a resistant reaction on Wilma, while others (e.g. no. 23) gave a susceptible reaction (Pl. 9, fig. 1). Wilma was, as already stated, added to the differential series. The number 6 has been retained for collections to which Wilma is susceptible, and the collection to which Wilma is resistant has been given the designation 6*x*. If Wilma, or some other wheat differentiating between races 6 and 6*x* becomes generally accepted as a differential host, race 6*x* will require a separate number in Straib's system. Race 6*x* differs in several respects from race 6; in particular, mature plants of various wheats are susceptible to race 6, but not to race 6*x* (see below p. 210).

Isenbeck, in Stakman *et al.* (1935), divided Gassner & Straib's race 6 into two with the aid of the wheat Golden Drop, but it is not known whether Golden Drop differentiates between races 6 and 6*x*. Gassner & Straib's race 6 is clearly a complex, and it will probably be necessary to add one or more new differential hosts to Straib's series to differentiate between the races in this complex. Neither Golden Drop nor Wilma is a good differential host. The reactions of Golden Drop, according to Gassner & Straib (1930), and those of Wilma, in the writer's experience, are both

strongly influenced by changes in environment and more suitable varieties are required.

(ii) *Races from hosts other than wheat*

One physiologic race was isolated from each of the hosts, other than wheat, from which collections were received. The race from barley gives the reactions on the differential hosts of races 45 and 46, which differ from each other only in spore germination characters (Straib, 1939*a*). The spore germination characters of the barley race isolated are those of race 46, to which it has therefore been assigned.

The reactions on the differential hosts of race 33, isolated from *Hordeum murinum*, agree in general with those quoted by Straib (1937*a*). Heines Kolben, however, gives a type 0+ reaction, instead of the type 2 reaction recorded by Straib (1937*a*) for race 33. This difference has been ignored, because the reactions of Heines Kolben to several races are rendered more resistant by slightly increased temperatures.

The race isolated from *Agropyron repens* agrees in its reactions on the differential hosts, with Gassner & Straib's races 28, 36 and 47. Races 28 and 36 are differentiated from each other by the susceptibility of the Gliesmarode collection of *A. repens* to race 28 and its partial resistance to race 36. Race 47 (isolated from wheat), with a straight uredospore germ tube at 15° C. differs from the race isolated by the writer which has a coiled germ tube at that temperature. The race isolated from *A. repens* in Britain could not be tested on the Gliesmarode collection of that species. It seems undesirable, on principle, to differentiate between physiologic races by their reactions on particular collections of a grass, especially when such collections are not included in the differential series. Race 36 has therefore been regarded as being included in race 28, and the British collections from *A. repens* have been referred to race 28.

All the differential hosts are immune to race *G*, which fails to infect any cereal or grass species tested, other than the original host, *Dactylis glomerata*. No race from *D. glomerata* appears to have been reported previously, and its status will be discussed later (p. 204). Race *M*, isolated from *Hordeum marinum*, is also new, no race from that host having been described previously. The reactions of race *M* on the differential hosts resemble those of the races from *H. murinum* and *Agropyron repens*. Neither *Hordeum murinum* nor *Agropyron repens*, however, is susceptible to race *M*, and *Triticum dicoccum* var. *tricoccum*, susceptible to races 28 and 33, is partially resistant to race *M*.

The effect of temperature and light intensity on the determination of physiologic races

The reactions of cereals to yellow rust are strongly influenced by environmental conditions. This is true even of the differential hosts selected by Gassner & Straib (1932) for the stability of their reactions. They showed that relatively high temperatures (e.g. 20° C.) increased the resistance of certain differential hosts, such as Webster, to some races, but decreased that of others, such as Holzapfels Früh. Low temperatures (e.g. 10° C.) increased the susceptibility of some differential hosts,

e.g. Heines Kolben, to certain races. Newton & Johnson (1936) showed that the duration for which a high temperature is maintained is important. It has been shown (Gassner & Straib, 1928; Wilhelm, 1931) that artificial light in winter increases pustule formation and that heavy shading decreases it, but the relative humidity subsequent to the first 48 hr. after inoculation does not appear to influence the reaction type appreciably (Gassner & Straib, 1932).

In ordinary glasshouses increased temperature and light intensity usually accompany each other. No measurements of light intensity were made in the present study, but the changes in reaction type accompanying changes in temperature, detailed in Table 2, may be attributable in part to changes in light intensity.

Blé rouge d'Ecosse and, more particularly, Webster show increased resistance to some races at high temperatures. Holzapfels Früh becomes more susceptible in hot weather to all races which it resists at low temperatures. Reactions on Vilmorin 23 are less affected by temperature changes, but this wheat becomes slightly susceptible to races 5, 7, 8 and 17 at 18° C., though it is highly resistant to them at lower temperatures. The type 0–1 reactions normally given by Carstens Dickkopf V and Spalding's Prolific to many races change to type 2–3 reactions at 18° C. In any one test Rouge prolifique barbu is more resistant to race 3 than to race 2, but the reactions to both races vary, susceptibility being greater at high temperatures than at low. The reactions of the other differential hosts are relatively unaffected by temperature. These results agree in general with those reported by previous investigators (Gassner & Straib, 1932; Küderling, 1936).

To supplement and confirm the results obtained in the glasshouse, experiments were made in a controlled environment chamber as described by Wilson (1937). The lighting system was a 400 W., fluorescent, high-pressure mercury-vapour electric-discharge lamp, and two 150 W. tungsten filament lamps. Inoculation and incubation were done in the usual manner, and the plants were then transferred to the chamber. The results of a test with races 6 and 8 are given in Table 3. They confirm

TABLE 3. *The effect of temperature and light on rust reactions in an environmentally controlled chamber at* 80% R.H.

Physiologic race ...	6				8			
Hours of light per day	12		18		12		18	
Temperature (° C.) ...	15	20	15	20	15	20	15	20
Holzapfels Früh	0–1	0–1	0	1	0–1	1–2	0	0
Carstens Dickkopf V	1–2	2–3	0	0	2	2–3	0	0

that at least some of the increased susceptibility of Holzapfels Früh and Carstens Dickkopf V to these races in warm weather is due to a temperature change. Increasing the duration of lighting from 12 to 18 hr. each day decreased the susceptibility of the two wheats at both temperatures, a result in agreement with that reported by Bever (1934). It is not clear why increased duration of artificial light increases resistance,

while increased daylight decreases it. The quality, as well as the intensity and duration of the illumination, is probably important.

Biotypes of certain races

The term 'biotype' has been used in several senses, but in recent studies on the rust fungi (Chester, 1946, p. 68; Stakman, 1947) it has meant strains of rusts which are genetically distinct, but which are sufficiently closely related to be included in a single physiologic race, and it will be so employed here. There have been few references to the existence of biotypes within physiologic races of *Puccinia glumarum*. Küderling (1936) isolated two biotypes of race 8. It is considered that Stakman's (1947) rule that a new physiologic race of a rust must not be established unless there is consistent difference in reaction type on one or more hosts at least as great as that between susceptible and mesothetic, or between mesothetic and resistant, should be applied to *P. glumarum*. If this is done, several races described by Straib (1939*a*) become biotypes of previously described races. Race 41 becomes a biotype of race 7, and races 40, 46 and 47, separated from races 20, 45 and 28 respectively only by spore germination characters, become biotypes of these.

Certain single spore lines which, under some conditions of temperature and light, gave reactions identical with those of the type collections of particular races but which, under slightly different conditions, gave divergent reactions, were studied in detail. They were found to belong to biotypes different from the type cultures of the races concerned. One such biotype of race 2, designated race 2, biotype *a*, two of race 6, designated race 6, biotypes *a* and *b*, and one of race 8, designated race 8, biotype *a*, were isolated. Race 2, biotype *a*, unlike the type culture of race 2, sometimes gave a type 2–3 reaction, instead of a type 0 reaction on Heines Kolben. Race 6, biotype *a*, usually gave a less susceptible reaction on Webster and a more susceptible reaction on Holzapfels Früh than the type culture of race 6, while race 6, biotype *b*, gave a more susceptible reaction on both those hosts than that given by the type culture. Race 8, biotype *a*, gave a type 0–1 reaction on Wilma at relatively low temperatures in place of the type 4 reaction given by the type culture of race 8.

Only a few of the collections of *P. glumarum* examined could be assigned to a particular biotype of a given race, hence no conclusions can be drawn concerning the host range or geographical distribution of particular biotypes, or their practical importance. The addition of extra differential hosts to Straib's series might well enable certain biotypes to be established as distinct physiologic races.

The host range and distribution of the wheat races in Great Britain

Host range

Of the collections from wheat determined on the differential hosts, 135 came from named varieties. A list of the races isolated from each variety is given in Table 4. The host range of each group of related races will be considered in turn. The host

TABLE 4. *The wheat varieties from which the wheat races of* Puccinia glumarum *were obtained*

Variety	Race	Variety	Race	Variety	Race	Variety	Race
Als	5	Guardsman	8	Red Standard	6	Victor	6
	6	Holdfast	8		8		7
	7		6	Renown	6		8
	8	Iron	8	Rouge	2	Vilmorin 27	2
Atle	8	Jubilegem	6	prolifique			3
	6		8	barbu			7
Bersée	2	Juliana	3	Scandia	8		8
	3		6	Spalding's	8	Vulgare pp.	6
Black winter	8		8	Prolific			8
emmer		Kolben II	6	Squarehead II	8	Warden	7
Chevalier	8	Little Joss	6	Squarehead's	6		8
Crown	8		7	Master		Webster	8
Desprez 80	2		17	Steadfast	5	Weibulls	
	3	Michigan	8		7	Standard	8
Desprez 80 ×		Amber		Strubes	8	Wilhelmina	6
Danish King	3	Norka	6	Dickkopf			7
Defiant	8		8	Sun × Wilma	8	Wilma	3
Fenland	8	Olympia	8	*T. dicoccum*	8		6
Wonder		Progress	8	var. *tricoccum*			8
Gartons 60	8	Red Man	8			Yeoman	6
Grey Spelt	6*x*	Red Russian	8				8

range of races 2 and 3 is restricted, and only nine collections of race 2 and ten of race 3 were examined. Race 2 was isolated from four varieties and race 3 from six, though for practical purposes these races are confined to Bersée, Desprez 80 and Vilmorin 27 in this country. One collection of race 2 came from Rouge prolifique barbu (one of the differential hosts) and one of race 3 from a hybrid, Desprez 80 × Danish King. Race 3 was also obtained from young plants of Wilma (two collections) and Juliana (one collection); these collections will be considered in a later section (p. 211).

Fourteen collections of race 7 were isolated from eight wheats, eight collections coming from Als and one from each of six other varieties. Of three collections from the closely related race 5, two were from Als and the other from Steadfast. The only other collection from Steadfast belonged to race 7.

Twenty-six collections of race 6 were obtained from twelve wheat varieties, notably Wilma. Of eleven collections from Wilma, seven belonged to race 6, two to race 8 and two, as already indicated, to race 3. Race 8, the commonest, was isolated seventy-one times, from thirty-seven wheat varieties. Although it is so common, race 8 attacks fewer varieties in the seedling stage than any other race, except for the very rare race 6*x* (p. 205). There is evidence, however, that the mature plant resistance exhibited by many wheats towards races more virulent on seedlings is not developed strongly towards race 8 (see p. 210). It is noteworthy that, of fifteen collections from Jubilegem, thirteen belonged to race 8, and that all the six collections from Scandia also belonged to that race.

Little is known about the host range of the other two races from wheat. The one collection of race 17 came from Little Joss, and the collection of race 6*x* came (in 1932) from Grey Spelt.

A given wheat variety may be attacked by one race early in the year and by another later in the season. Such double attacks occurred on certain varieties at Owstwick, Yorkshire, in 1946 and 1947 (see Table 5). The only collections of race 3 isolated

TABLE 5. *Changes during the growing season in the races attacking certain wheats at Owstwick, Yorks*

Year	Variety	Date	Race	Date	Race
1946	Wilma	24 April	3	24 June	6
	Vilmorin 27	24 April	3	24 June	8
1947	Wilma	7 May	3	3 June, 16 July	6
	Juliana	3 June	3	16 July	6

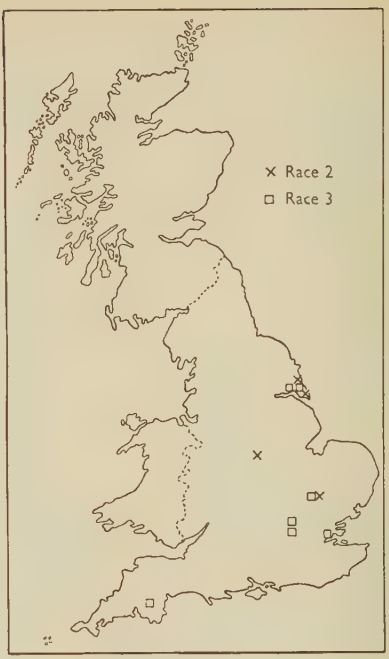
from Wilma and Juliana came from Owstwick early in the year, though race 3, not race 8, was the race usually isolated from Vilmorin 27. Experiments which will be considered later (p. 210) indicated that mature plants of Wilma resist race 3. Dr I. F. Storey informed the writer that in 1946 the infection intensity curve for Wilma had two peaks, while those for other heavily infected varieties, e.g. Jubilegem, had one. It seems probable that Wilma was first attacked by race 3, but became increasingly resistant to it, and was later attacked by race 6. The change on Juliana probably had similar causes to that on Wilma. Although Juliana exhibits mature plant resistance to race 6, it is less pronounced than that developed to race 3 (see p. 209). No explanation of the change on Vilmorin 27 can be given.

Geographical distribution

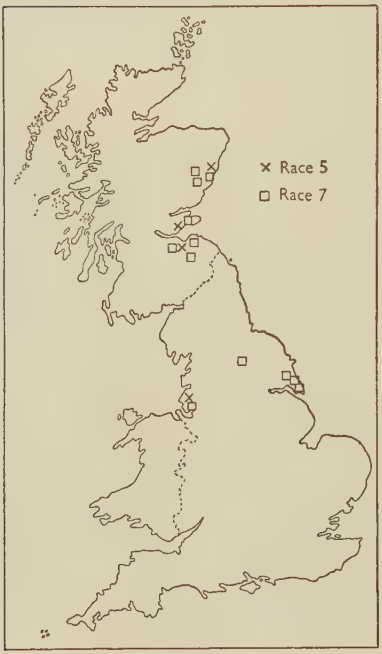
The localities from which the more important wheat races were isolated are given in Text-fig. 1, related races being grouped together. It should be borne in mind that the distribution of all collections received is very uneven (Text-fig. 1*a*). The apparent restriction of races 2 and 3 (Text-fig. 1*b*) to central and southern England is probably because they are restricted to a few wheat varieties (in particular, Bersée and Desprez 80). No collections from either Bersée or Desprez 80 were received from Scotland, but Dr E. Gray of Aberdeen found yellow rust on Desprez 80 in Aberdeenshire in 1943, and no known British races, other than races 2 and 3, attack that variety. Gassner & Straib (1943*a*, *b*) and Straib (1935*a*, 1937*a*, 1939*a*) isolated races 2 and 3 from Germany, Holland, France, Belgium and the Balkans: it is probably significant that both Bersée and Desprez 80 are French wheats.

The restriction of races 5 and 7 (Text-fig. 1*c*) to the north is not easy to explain, for race 7, the commoner of the two races, has been isolated from a number of wheats, some of which, e.g. Squarehead's Master, are widely grown in southern England. Both races have, however, been isolated most frequently from Als, a wheat grown mainly in the north, and the distribution of races 5 and 7 may depend on the

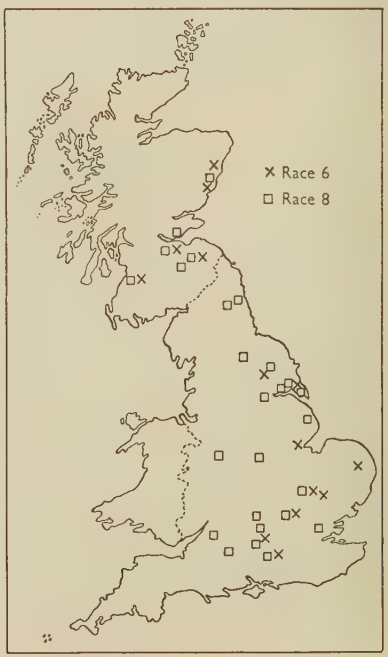
Text-fig. 1 *a*.



Text-fig. 1 *b*.



Text-fig. 1 *c*.



Text-fig. 1 *d*.

Text-fig. 1. Localities from which the principal races from wheat were obtained during 1945–8

cultivation of Als. Gassner & Straib isolated races 5 and 7 from Germany, Sweden, Holland and Czechoslovakia, and Als is a Danish wheat.

Races 6 and 8 were both generally distributed throughout Great Britain (Text-fig. 1 *d*), probably because they can attack a wide range of hosts. Gassner & Straib isolated races 6 and 8 from German and Dutch collections. Race 6 also occurred in England and Bulgaria. The single collection of race 17 came from Hampshire: Gassner & Straib isolated this race from only four German collections. The culture of race 6*x* was derived from a sample collected at Cambridge by Miss M. Cudmore in 1932, and maintained in culture subsequently.

The barley race was probably generally distributed, but appeared to be frequent only in experimental stations. A possible explanation is that in such stations winter barleys on which the rust can over-winter, are frequently grown, whereas nearly all important commercial barley varieties are spring grown. Too few collections were received from grasses to enable any conclusions to be drawn concerning the geographical distribution of the grass races.

The reactions of certain grasses to different races of yellow rust

Many investigators have recorded the presence of yellow rust on grasses in the field, or have obtained pustules on artificially inoculated plants. The results of inoculating grasses with specific physiologic races have been recorded by Hassebrauk (1932), Straib (1935*b*) and Newton & Johnson (1936). They showed that certain races can attack a rather wide range of grasses as seedlings.

All races isolated by the writer were tested on several common grass species. The tests were made in the same way as those on the differential hosts except that the second or third leaf was inoculated, the first usually being too small. At least six plants were inoculated, because of the variation in reaction found even within a single collection of some grass species. The results were recorded according to the scale given by Straib (1935*b*), and are summarized in Table 6. Tests were carried out in each of the years 1946, 1947 and 1948.

Agropyron caninum proved resistant to all races tested. Most plants of *A. repens*

Table 6. *The reactions of certain grasses to the physiologic races isolated*

Usual host ...	Wheat								Barley	*Hordeum murinum*	*Agropyron repens*	*Hordeum marinum*	*Dactylis glomerata*
Physiologic race ...	2	3	5	7	17	6	6*x*	8	46	33	28	*M*	*G*
Agropyron caninum	0	—	—	0	—	0	0	0	—	—	—	—	—
A. repens	i–0	i–0	i–0	i–0	i–0	i or 1–2	i or 1–2	i or 1–2	i–0	i–0	i or 3–4	0	i
Bromus erectus	i	i	i	i	—	i	i	i	i	i	—	—	i
B. mollis	i	i–0	i–0	i–0	—	0	i	i–0	i	i–0	—	—	i
B. sterilis	0 or 1–2	0 or 1–2	0	0	—	0	i	0 or 1–2	1–2	1–2	—	—	i
Dactylis Aschersoniana	i	i	i	i	i	i	i	i	i	i	—	—	—
D. glomerata (American)	i	i	i	i	i	i	i	i	i	i	i	i	4 or 0
D. glomerata (Danish)	i	i	i	i	—	i	i	i	i	i	i	—	4 or 0
Hordeum marinum	0 or 3–4	0 or 3–4	0 or 3–4	0	0	0 or 3–4	0	0 or 3–4	0 or 3–4	0 or 3–4	0	4	—
H. murinum	i–0	i–0	i–0	i–0	i–0	i–0	i–0	i–0	i or 2	3–4	i–0	i–0	i
H. nodosum	i	i	i	0	—	i	i	i	i	i	—	—	i
Lolium perenne	i	i	i	i	i	i	i	i	i	i	i	—	i–0

were resistant not only to the races from other hosts but also to race 28, only about one plant in fifty being susceptible to that race. This explains why yellow rust is uncommon on *A. repens*, though it has been reported on it from a number of widely scattered localities. Pustules were obtained on single plants of *A. repens* with races 6, 6*x* and 8. No pustules were obtained with any race on *Bromus erectus* Huds. or *B. mollis* L., but races 2, 3 and 8 from wheat, race 46 from barley and race 33 from *Hordeum murinum* produced pustules on *Bromus sterilis*.

Dactylis glomerata proved to be immune to all races of *Puccinia glumarum* tested, other than race *G*, the cocksfoot race, which differs greatly from all other known races. Yellow rust has been reported from *Dactylis glomerata* by Klebahn (1898), Sampson (1924), Guyot (1932) and Viennot-Bourgin (1934), but no specific physiologic race of *Puccinia glumarum* on cocksfoot has been reported previously. Race *G* could not be tested on *Dactylis Aschersoniana*, but this species, like *D. glomerata*, was immune to all other races. With several strains of *D. glomerata* from Aberystwyth (kindly supplied by Mr Crespin) the proportion of susceptible plants varied from one strain to another.

Hordeum marinum gave a uniformly susceptible reaction to race *M*, isolated from that host. However, some plants gave a type 3–4 reaction, others a type 0 reaction, when inoculated with other races. *H. murinum* appeared immune or highly resistant to all races, except race 33 from *H. murinum* and race 46, the barley race. Most of the plants tested were susceptible to race 33, but only a few to race 46.

The results obtained agree in general with those of previous workers, though there are some discrepancies. Divergent results by different investigators in testing a given grass species are to be expected because of the genetical diversity within most species. Wheat races of yellow rust might over-winter on grasses in Britain. Cereal races were isolated from grasses in Germany by Becker & Hart (1939), but no cereal race has been isolated from any grass in this country. Of the three grasses on which infection by wheat races was obtained, *H. marinum* is a purely maritime species, and *Agropyron repens* and *Bromus sterilis* were only slightly susceptible to wheat races. Consequently it is improbable that wheat races over-winter on grasses in Britain.

Uredospore characters

Experiments on spore germination

(i) *Introduction*

Straib (1939*c*, 1940) studied the morphology and physiology of spore germination in *Puccinia glumarum*. He extended the experiments of Wilhelm (1931) and Stroede (1933) on the effect of environmental factors on spore germination, and also (Straib, 1937*a*, 1939*a*) demonstrated differences in spore germination characters between different races. Good correlations were obtained between the maximum temperatures for germination and those for infection and survival of the mycelium in the leaf.

(ii) *Materials and methods*

The methods employed were essentially those described by Wilhelm (1931) and Straib (1940). Spores from plants kept in a cool place under bell jars for 24 hr. were removed from the leaf by means of a sterile camel-hair brush and streaked across the surface of sterile agar in a Petri dish. Filter-paper, moistened with sterile water, was placed in the lid of each dish. The germinating spores were observed through the bottom of the dish. The dishes were incubated in darkness at various temperatures between 5 and 25° C.

The results obtained in early spore germination experiments were very irregular, and varied widely from one experiment to another. Several factors were found to influence spore germination.

(1) The time of year. Spores germinated more rapidly and completely in summer than in winter (cf. Straib, 1940). In December and January it was impossible to obtain more than 70% germination at 10° C., compared with 100% germination normally obtainable at that temperature. This was probably due to the effect of low light intensities on the plant and hence, indirectly, the rust.

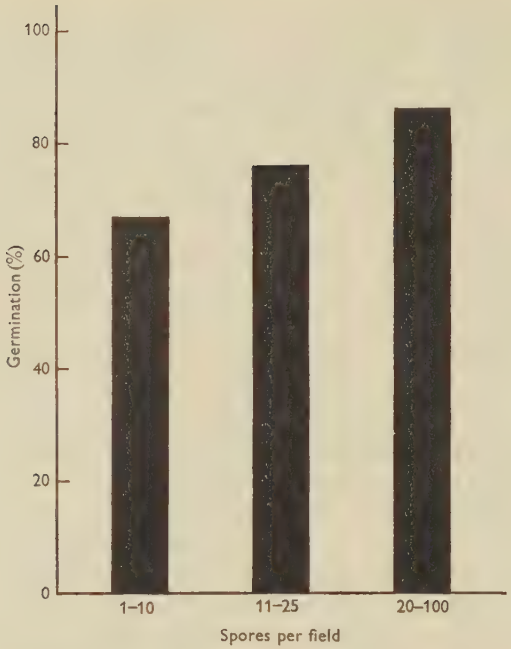
(2) As noted by Becker (1928), spores which had become wetted germinated less well than those which had not. Consequently the brush employed for the transfer of the spores was not wetted by drops of condensation on the leaf or the agar.

(3) The germination percentage was higher when the spores were densely aggregated than when they were separated. The results of an experiment illustrating this are shown in Text-fig. 2. One hundred fields of the low power of the microscope were examined, and the results were grouped into three classes according to the number of spores present in the field. The differences in percentage germination between the classes were statistically significant. The nature of the stimulus is unknown, but Straib (1940) has shown that various substances stimulate the germination of the uredospores of *P. glumarum*.

(4) The type of agar employed is important. Agar from New Zealand gave more rapid and complete germination than the 'Difco' bacteriological agar usually employed (see Table 7). The stimulating substance present in the New Zealand agar was removed by washing in running water for 24 hr. The nature of the stimulant is not known.

TABLE 7. *The effect of the type of agar employed on the germination of the spores of race* 8

Temp. (° C.)	Substrate	Hours after inoculation 6	12	24	48
13	New Zealand agar unwashed	100	—	—	—
	New Zealand agar washed	80	96	99	100
	Difco agar unwashed	80	98	99	98
	Difco agar washed	80	97	99	97
20	New Zealand agar unwashed	11	36	99	100
	New Zealand agar washed	0	0	0	80
	Difco agar unwashed	1	2	44	81
	Difco agar washed	0	0	11	94



Text-fig. 2. The relationship between the density of the spore deposit and the percentage germination of spores of race 6 after 12 hr. incubation at 13° C.

(iii) *Results*

In July 1946 spores from barley (race 46), wheat (race 6*x*) and *Dactylis glomerata* (race *G*) were tested simultaneously at four different temperatures. Some of the results are given in Table 8. A second similar experiment confirmed these results. Race *G* is remarkable in that its spore germination optimum is approximately 22·5° C. The optimum for all other known races is approximately 10–13° C.

TABLE 8. *The effect of temperature on the germination of spores of races* 6*x*, 46 *and G*

Hours after inoculation	Race	Temperature (° C.) 5	17	22·5	25
12	6*x*	90	20	3	0
	46	80	2	0	2
	G	0	4	50	38
48	6*x*	98	13	4	0
	46	96	50	2	2
	G	2	16	94	88

Exactly reproducible results could not be obtained in spore germination tests, even when all the factors mentioned above were taken into consideration. Hence the comparatively small differences in spore germination between one wheat race or

biotype and another, which were observed, could not be regarded as significant unless they occurred consistently in a number of experiments. The results obtained in five experiments in the spring of 1948 are summarized in Table 9. Races 2 and 3

TABLE 9. *The effect of temperature on the germination of the spores of the wheat races on New Zealand agar. The results represent the mean of five experiments*

	Temperature (° C.)				
Race	5	10–13	15–18	22·5	25
2	64	78	29	1	0
3	53	81	28	1	0
5	94	99	83	0	0
6	81	100	98	0	0
6*x*	82	99	79	2	1
8	87	100	99	7	0

germinate more slowly than do the other wheat races, and have a lower temperature maximum (Pl. 9, figs. 2*a*, *b*), while race 8 germinates particularly rapidly. Race 6*x* germinates rather slowly, but not as slowly as races 2 and 3, while races 5 and 6 are intermediate between races 8 and 6*x*. Other experiments showed that race 7 behaves like race 5, and race 17 like race 6*x*. The maximum temperature for spore germination and the rate of germination are correlated (see Table 9). The races germinating most rapidly have the highest maximum temperatures for spore germination. The results obtained are in general agreement with those of Straib (1940).

The morphology of spore germination was also studied. Apparent anastomoses between germ tubes and various abnormal structures often occurred 24–48 hr. after inoculation. No evidence was obtained of an actual fusion, and these phenomena were observed only when hyphae were moribund and their nuclei were no longer stainable with haematoxylin after fixation in acetic alcohol. Rodenhiser & Hurd-Karrer (1947) observed true fusions between living germ tubes in certain other rust species.

Occasionally a germ tube swelled at the tip, forming a secondary vesicle, which sometimes produced one or two hyphae rather thicker-walled than the original germ tube. Like the similar bodies observed by Straib (1940), they may be homologous with substomatal vesicles. No secondary vesicles formed as a result of hyphal fusion were observed (cf. Hurd-Karrer & Rodenhiser, 1947).

It is doubtful whether the differences in spore germination physiology between one race and another are normally of any importance in the epidemiology of yellow rust. Race *G*, however, is probably an exception to this general rule. The high optimum temperature for the germination of spores of race *G* may be related to the appearance of *Puccinia glumarum* on *Dactylis glomerata* in September, instead of in spring or early summer, when yellow rust appears on other hosts.

Measurements of spore size, with special reference to the status of race G

Twenty dry spores of each of the races 6*x*, 46 and *G* were measured. The results (Table 10) indicate that the spores of race *G* are significantly smaller than those of other races. Too few spores were measured for firm conclusions to be drawn. Differences in spore size have not previously been described in *Puccinia glumarum*, though they have in *P. graminis* (Levine, 1923).

TABLE 10. *The spore dimensions of races* 6*x*, 46 *and G*

Race	Length (μ)	Standard error (μ)	Breadth (μ)	Standard error (μ)
6*x*	24·5	0·54	18·4	0·38
46	23·8	0·60	18·5	0·28
G	20·2	0·52	17·2	0·29

Statistical comparison of the spore dimensions of races 6*x*, 46 *and G*

	Spore length		Spore breadth	
Comparison	*t*	*P*	*t*	*P*
Races 6*x* and 46	0·866	0·5 to 0·3	0·212	0·9 to 0·8
Races 6*x* and *G*	5·73	<0·001	2·49	0·02
Races 46 and *G*	4·57	<0·001	3·23	0·01 to 0·001

The statistical method employed was that given by McCallan & Wilcoxon (1932).

Race *G*, from *Dactylis glomerata*, differs in many respects from all races previously described. In addition to the difference in spore size, all cereals and grasses tested (except *D. glomerata*), including *Triticum dicoccum* var. *tricoccum* and Fong Tien barley, previously considered to be susceptible to all races of yellow rust, are immune to race *G*. No other known race of *Puccinia glumarum* attacks *Dactylis glomerata*. Its spore germination optimum is 22·5° C., instead of the usual 10–13° C., and it is an autumn, not a spring, species. Teleutospores of race *G* were not obtained, but if future investigations confirm that there are morphological differences between race *G* and other races, it may be necessary to establish that race as a distinct variety of *Puccinia glumarum*, or possibly even as a new rust species.

THE REACTIONS OF CERTAIN COMMERCIAL WHEATS TO DIFFERENT RACES OF YELLOW RUST

Particulars concerning the relative susceptibility to yellow rust of wheats commonly cultivated in Britain were provided by Dillon Weston (1944). No data have been published, however, concerning the susceptibility of such wheats to specific races of *P. glumarum*.

With most cereal rusts, a variety susceptible as a seedling to a particular rust race may become resistant to that race later. With yellow rust such resistance is known as field resistance (Straib, 1939*b*); early investigators (Gassner & Straib, 1929;

Radulescu, 1933; Gassner & Kirchoff, 1934) considered that high summer temperatures were the most important cause of field resistance. Field resistance may also develop as the crop matures, regardless of external conditions, and is then termed mature plant resistance. (Here the term mature plant resistance will not be used to describe field resistance caused by factors other than the maturity of the plant.) Mature plant resistance to *P. glumarum* was first demonstrated by Küderling (1936), who showed that a variety might develop it to one race while remaining susceptible to another: high temperature resistance was usually developed equally towards all races. Straib (1939*b*) confirmed these conclusions, and showed that some varieties exhibited high temperature resistance to particular races at all stages, others only when mature. The object of the experiments to be described was to determine whether commonly grown British wheats develop field resistance to the races of yellow rust isolated from wheat in this country.

Tests on seedlings

The methods employed were those used for the testing of rust samples on the differential hosts (p. 189). Tests were made in 1947 and 1948 on fifteen wheat varieties. Seed of Rampton Rivet, Bersée, Scandia, Joss 4, Squarehead's Master, Yeoman, Wilma, Holdfast, Jubilegem, Juliana, Als, Vilmorin 27 and Steadfast was kindly supplied by the National Institute of Agricultural Botany, Cambridge. Seed of Wilhelmina and Desprez 80 was obtained from the collection at the Botany School, Cambridge. Tests were made with single spore cultures of all races available in the spring and autumn of 1947 and 1948. In no experiment was the average temperature below 11° C. or above 17° C. The results agreed well and are summarized in Table 11.

All the wheats tested were highly resistant to race 46 (from barley), race 33

TABLE 11. *The reactions of certain commercial wheats as seedlings to different races of* Puccinia glumarum

Usual host ...	Wheat								Barley	*Hordeum murinum*	*Agropyron repens*
Physiologic race ...	2	3	5	7	17	6*x*	6	8	46	33	28
Wilhelmina	3–4	4	4	4	4	3–4	4	4	0 0	0 0	0 0
Rampton Rivet	3–4	4	0–1	0–1	0–1	0–1	0	0–1	0	0–1	0
Bersée	3–4	4	0–1	0	0	0	0	0	0 0	0 0	0 0
Scandia	3–4	3–4	3–4	4	3–4	0–3	4	4	0 0	0 0	0 0
Joss 4	3–4	4	4	3–4	4	3–4	3–4	3–4	0 0	0 0	0 0
Squarehead's Master	4	4	3–4	4	4	3–4	3–4	3–4	0 0	0 0	0 0
Yeoman	3–4	3–4	4	3–4	0–1 or 3–4	0–1 or 3–4	3–4	3–4	0 0	0 0	0 0
Wilma	3–4	3–4	4	4	3–4	0–2	4	4	0 0	0 0	0 0
Desprez 80	4	4	0	0	0	0	0	0	0 0	0 0	0 0
Holdfast	3–4	4	3–4	2–3	1–3	0	0–2	1–3	0 0	0 0	0 0
Jubilegem	3–4	4	3–4	3–4	3±	0–1 or 4	4	4	0 0	0 0	0 0
Juliana	0–4	0–4	0–4	0–4	0–4	1–4	0–4	0–3	0 0	0 0	0 0
Als	0 or 3–4	0 or 3–4	3	4	0–1 or 3–4	0–1 or 3–4	0–1 or 3–4	0–1 or 3–4	0 0	0 0	0 0
Vilmorin 27	3–4	2–3	0–1	0	0	0	0–1	0–1	0 0	0 0	0 0
Steadfast	4	4	4	4	4	4	3–4	3–4	0 0	0 0	0 0

(from *Hordeum murinum*) and race 28 (from *Agropyron repens*). By their reactions to the wheat races the wheats could be separated into groups. Wilhelmina, Joss 4, Squarehead's Master and Steadfast were susceptible to all races from wheat, and Scandia, Wilma and Jubilegem to all except 6*x*. Yeoman was susceptible to all races except 6*x* and 17, and Holdfast to all except 6*x*, 17, 6 and 8. The reactions of Juliana to all races were extremely variable. This wheat is clearly a mixture of several strains differing in their reactions to *Puccinia glumarum*. Als was susceptible to races 5 and 7, but some plants gave a susceptible reaction, and others a very resistant one, when inoculated with any other wheat race. Rampton Rivet, Bersée, Desprez 80 and Vilmorin 27 were highly resistant to all races except 2 and 3.

An experiment (Table 12) was done in May 1947 at an average temperature of 21·25° C., to determine the effect of high temperatures (and high light intensities) on the reactions to certain races of twelve of the commercial wheats. Almost all hosts were unusually resistant. Yeoman, Holdfast and Juliana were resistant to all races tested and Wilhelmina to all except 17. Scandia resisted all races except 7 and 8, Joss 4 all except 17 and 6*x*, and Jubilegem all except 6 and 8. Bersée resisted race 2. Rampton Rivet was unusual in losing its resistance at lower temperatures to all races except 2 and 3, and it was susceptible to all races except 6*x*. The significance of these results will be discussed later.

TABLE 12. *The reactions of certain commercial wheats as seedlings at* 21·25° C.

Physiologic race ...	2	5	7	17	6*x*	6	8
Wilhelmina	0–1	2	0–1	3	0–2	0–1	0
Rampton Rivet	4	3–4	3	3–4	1–2	3±	3–4
Bersée	2	0	0	0	0–1	0	0
Scandia	0	1	4	1–3	0–1	1	3
Joss 4	0–3	1–2	0–2	3	2–3	0–2	0–1
Squarehead's Master	0–1	2	0–1	3	1–2	0	0–1
Yeoman	0 or 2–3	1–3	2	3	0–1	2–3	2
Wilma	2–3	4	3	3	2	3	3
Desprez 80	4	1–3	0	0–1	0–1	0	0
Holdfast	0	2–3	2	1	0–1	0	0
Jubilegem	2–3	2	2	0	0–1	3	3
Juliana	0	0–1	0	0	0	0	0

Tests in the field on mature and semi-mature plants

(i) *Materials and methods*

The various methods employed to produce artificially induced rust epidemics in the field have been reviewed by Cherewick (1946). In my experiments a modification of the method developed by Gassner & Straib (1931) was used. Fifty seedlings of a susceptible variety, grown in a box, were inoculated with a scalpel and incubated in the usual manner. When spores were abundant, they were scraped off the leaves with a scalpel and a suspension was made in 125 ml. of 0·1% plain agar. (It is difficult to obtain a satisfactory suspension of rust spores in water, because they

float, but a suspension is readily made in the viscid agar solution.) The suspension was placed in a large sterilized atomizer and sprayed over the experimental plots, care being taken to ensure even distribution.

The plots were inoculated in the late afternoon, and each plot was watered immediately before with a sprinkler. When possible, inoculations were made on cool, dull, still days.

In each of the seasons 1946–7 and 1947–8 the following twelve varieties were sown: Wilhelmina, Rampton Rivet, Bersée, Scandia, Joss 4, Squarehead's Master, Yeoman, Wilma, Desprez 80, Holdfast, Jubilegem and Juliana. The experimental plots were at the Botany School Field Station, Cambridge, on a gravelly clay soil overlying chalk. A distance of at least 10 yd. separated any two plots. Within each plot, six 1 ft. drill lengths of each variety were sown. The seventy-two drill lengths in each plot were distributed in the form of a modified Latin square. The pattern was such that each variety occurred once on each of the twelve rows of six drill lengths, and once in each position in each row. Each plot was surrounded by a double row of winter oats to protect the wheat near the edges of the plot from drying winds. The plots were sown in October. All seed was dressed with an organic mercurial before sowing.

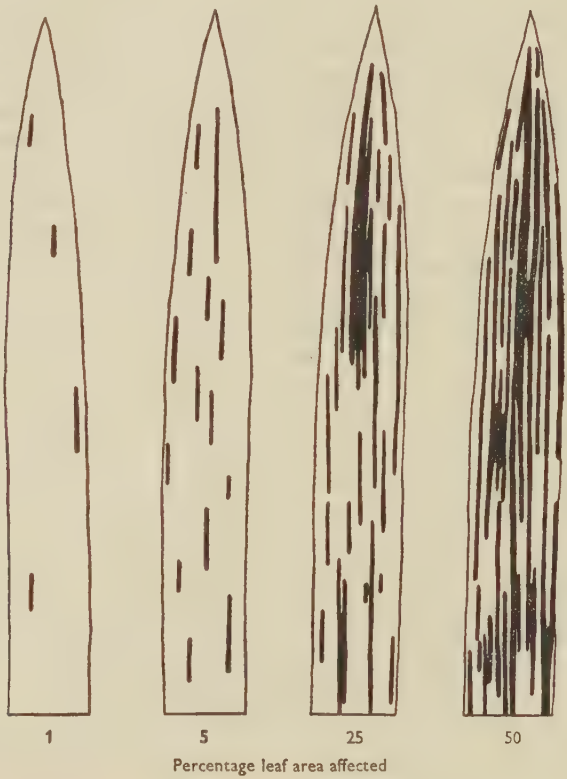
When the rust epidemic developed, it was necessary to record the amount of yellow rust present on each drill length by means of a method both precise, objective and reasonably rapid. Methods for estimating the intensity of yellow rust attack have been described by Ducomet & Foëx (1928), Dillon Weston (1929), Isenbeck (1931) and Straib (1939*b*), but the method employed by the writer was based on the work of a Committee of the British Mycological Society (Moore, 1943; Anon., 1948). Of the general methods recommended, the 'percentage area method' is most applicable to *P. glumarum*. This measures the intensity of attack, i.e. the proportion of the total leaf area affected by the disease, but not the prevalence, i.e. the proportion of plants in the crop showing symptoms. The 'percentage area method' was applied to the cereal rusts by Melchers & Parker (1922) and Tehon (1927).

Each 1 ft. drill length was examined, and the amount of rust present was estimated by eye on the following scale, according to the approximate percentage of the leaf area affected by rust: 1, 5, 25, 50, 75.

Standard area diagrams, similar to those employed by Grainger (1947) for use with *Erysiphe graminis*, were constructed to aid the assessments (see Text-fig. 3). To obtain the average percentage leaf area affected in each variety in each plot, the mean of the values estimated for each of the six drill lengths was calculated. In making estimations, the term 'leaf' was taken to include both blade and sheath, but not dead leaves or parts of leaves. The presence of rust on the ears, dead rusted foliage, and/or teleutospores was noted separately.

The assessment of necrosis caused by rust presents considerable difficulties as it can have other causes. Furthermore, the amount of necrosis produced by *Puccinia glumarum*, in relation to the amount of pustule formation, varies considerably. For

example, much necrosis occurs on Jubilegem, but very little on Wilma, both varieties being equally susceptible to yellow rust in the field. Necrotic areas on a leaf were included as part of the affected leaf area only when they surrounded rust pustules and were obviously caused by the fungus. This method was adopted as a practicable compromise, and is not regarded as ideal.



Text-fig. 3. Provisional standard diagrams for the estimation of the intensity of yellow rust attack.

The accuracy of field estimations made by the 'percentage area method' was checked in July 1947. Estimations of rust intensity were made on each of nine wheat varieties in a variety trial. A large sample of leaves was collected from each of four of these varieties, and fifty leaves of each variety were measured with a planimeter to determine the percentage leaf area affected. The results are given in Table 13, and confirm that the method is reasonably accurate and objective.

Observations were made regularly, either monthly or fortnightly, from the time when yellow rust first appeared until the crop ripened. The incidence of other

parasites, e.g. *Erysiphe graminis* and *Puccinia triticina*, was never sufficiently great to interfere with the results.

TABLE 13. *Measured and estimated values of the percentage leaf area affected in certain wheats at Owstwick, Yorks, in July* 1947

Variety	Percentage leaf area affected	
	Estimated	Measured
Jubilegem	55	47·5
Wilma	55	45·3
Scandia	13	13·7
Als	5	2·0

(ii) *Results*

In 1947 inoculations were made on 31 March, and at fortnightly intervals until 20 June. The weather was damp during April, and rust was present on all plots by the end of that month. The plants were still in the rosette stage and the reactions were, in general, the seedling reactions of the varieties concerned. The growth of the wheat on some plots was poor, owing to the shallowness of the soil, and on such plots the rust died out completely as a result of hot weather at the end of May. The rust attack on the plots infected with races 6, 6*x* and 7 maintained itself until the middle of July, when the onset of a prolonged spell of very hot, dry weather killed it.

The plots were given a dressing of farmyard manure and wood ash in the autumn of 1947, and the following winter, unlike that of 1946–7, was mild. As a result, the plants were past the rosette stage when spraying at fortnightly intervals commenced on 18 March 1948. March and April were very dry, and no rust was observed until 10 May. The weather was cool in May and June, and the attack developed rapidly, except on the plot inoculated with race 7, which made very poor growth, and was discarded.

TABLE 14. *The intensity of certain races of* Puccinia glumarum *on twelve wheats in* 1947 *and* 1948

(The results are expressed as the rust-infected percentages of the total leaf area.)

Physiologic race	2	3	5	7	6*x*		6		8	Control
Year	1948	1948	1948	1947	1947	1948	1947	1948	1948	1948
Wilhelmina	1·5	2·5	3·5	4	0	1	7	7·5	5·5	0
Rampton Rivet	0·5	0·1	0	0	0	0	0	0	0	0
Bersée	1·5	0·1	0	0	0	0	0	0	0	0
Scandia	2·5	0·5	11·5	6	0	0	5	30	8·5	0
Joss 4	0·1	0·5	0·5	6	1	0	6	0·5	0·5	0
Squarehead's Master	1	1	0·5	6	4	0·5	4	1·5	1·5	0
Yeoman	1·5	0·1	0·5	2	0	1	3	0·1	1·5	0
Wilma	0·5	0·5	14·5	10	0	0	21	26	7	0
Desprez 80	6·5	33·5	0·1	0	0	0	0	0	0	0
Holdfast	0·5	0·1	0	0·5	0	0·1	0·1	0·5	1	0
Jubilegem	0·1	0·5	0	0·5	0	0	0·5	0	11·5	0
Juliana	0·5	0	0·1	0·5	0	0	1	0·1	0·5	0

The assessments were made in 1947 on 10 July and in 1948 on 23 June.

The results of assessments made on 10 July 1947 and 23 June 1948 are given in Table 14 (see also Pl. 10, figs. 1, 2). Race 3 gave rise to a very heavy infection on Desprez 80 and a slight to moderate infection on Wilhelmina, but attacked only slightly the other variety under trial. Race 2 behaved similarly to race 3, but attacked Desprez 80 rather more lightly, and Bersée rather more heavily. Races 5, 6 and 7 attacked Wilma and (in 1948) Scandia heavily, and Wilhelmina and (in 1947) Squarehead's Master and Joss 4 moderately heavily. Other varieties were, at the most, only slightly attacked by those three races. No variety was markedly susceptible to race 6*x* in 1947 or 1948, but race 8 infected Jubilegem heavily, and Scandia, Wilma and Wilhelmina moderately, though other varieties were attacked only slightly or not at all. The control plot was free from rust in 1948, and was only very slightly attacked in 1947, when the intensity of attack never exceeded 1%. Check samples of rust were taken in May and June in both years from all affected plots, and invariably the race with which the plot had been inoculated was recovered in the sample.

(iii) *Discussion*

A comparison of the data given in Tables 11, 12 and 14 reveals that when a wheat is resistant as a seedling at 11–17° C. to a given race, it is always resistant to that race when a mature plant. The varieties highly susceptible to particular races in the field, e.g. Desprez 80 to race 2 and Wilma to races 5, 6 and 7, were susceptible, as seedlings, to those races, even at relatively high temperatures. Many varieties exhibited field resistance to one or more physiologic races. The field resistance of Rampton Rivet, Wilma and Jubilegem to race 2 (and probably race 3), of Jubilegem to race 6 (and probably other races) and perhaps that of Yeoman and Holdfast to certain races, cannot be explained by high temperatures and is almost certainly true mature plant resistance. The field resistance of Joss 4, Squarehead's Master and, to a lesser extent, Wilhelmina, to all races tested may have been due to high temperatures, the maturity of the plants, or both. The results confirm the conclusion drawn by Straib (1939*b*) that high temperature resistance, when developed, is usually, unlike mature plant resistance, exhibited towards all races of yellow rust.

The rust races isolated most frequently from collections of a particular variety were usually the races attacking that variety most heavily in the field trials. This was particularly so with varieties highly susceptible to yellow rust. Desprez 80 is highly resistant, even as a seedling, to all races except 2 and 3, and all the collections from Desprez 80 belonged to one of those two races. The Desprez 80 employed in the field trials was of the normal short-strawed type, not of the long-strawed type with which the normal type is mixed in most crops, and which is resistant, when mature, to yellow rust. Brooks (1944) stated that Desprez was much more heavily attacked by yellow rust in the south of England than in the north. This is probably related to the distribution of races 2 and 3 (see p. 197), which apparently occur only in the southern half of England.

Scandia, although showing field resistance to races 2 and 3, is susceptible when

mature to races 5, 6, 7 and 8. No explanation can be given for the fact that all the eight collections from Scandia belonged to race 8. Wilma developed mature plant resistance to races 2 and 3, but was particularly susceptible in the field trials to race 6. Seven collections from Wilma belonged to race 6, and two to race 8, to which it was moderately susceptible when mature. It is not known why no collections from Wilma belonged to races 5 or 7, since in the field trials Wilma was more susceptible to those races than to race 8. The strong mature plant resistance of Wilma to race 3 explains the replacement of that race by race 6 at Owstwick (p. 197).

Jubilegem, showing field resistance to all races except race 8, yielded thirteen collections of that race. The other two collections from Jubilegem, which were assigned to race 6, were gathered early in the year, from plants probably still giving seedling reactions to yellow rust. Jubilegem, though resistant as a mature plant to race 6, is susceptible as a seedling. Of the six collections from Wilhelmina, three belonged to race 6, two to race 8 and one to race 7. These were the three races attacking Wilhelmina most heavily in the field trials. The other seven varieties were not heavily attacked by any race in the field trials, and relatively few collections were received from them. It may be noted that the mature plant resistance developed by Juliana to race 3 is more strongly developed than that towards race 6, which gives a possible explanation of the replacement of race 3 by race 6 at Owstwick (p. 197).

Dillon Weston (1944) stated that the twelve varieties under consideration could be arranged as follows in descending order of susceptibility to *Puccinia glumarum*: Desprez 80, Wilma, Squarehead's Master and Wilhelmina, Scandia, Bersée and Juliana, Jubilegem, Holdfast and Yeoman, Little Joss, Rivet. If these wheats are arranged according to the susceptibility of each variety to the race attacking it most severely in the 1947–8 field trials, the order is: Desprez 80, Scandia, Wilma, Jubilegem, Wilhelmina, Yeoman, Squarehead's Master, Bersée, Holdfast, Juliana, Joss 4 and Rampton Rivet. This order agrees well with that given by Dillon Weston (1944) except that he reported that Scandia and Jubilegem were relatively more resistant than they were in the field trials. The susceptibility of both these wheats is, however, strongly influenced by temperature changes, and field observations indicate that the intensity of yellow rust attack on them varies greatly from year to year.

It may be claimed that field trials, such as those described, reasonably indicate the behaviour of a wheat variety under the climatic conditions of the trial in a year in which yellow rust is abundant. If a variety is resistant as a seedling to a particular race of *P. glumarum*, it is resistant to that race as a mature plant. There are apparently no exceptions to this. If a variety is susceptible as a seedling to a particular race, it may or may not be susceptible under field conditions as a mature plant. If it is not fully susceptible as a seedling, or if it shows seedling resistance at relatively high temperatures, it is improbable that it will be severely attacked in the field. If a variety is fully susceptible, as a seedling, to a particular race, even at relatively high temperatures, it may or may not develop field resistance to that race when mature.

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EXPLANATION OF PLATES 9 AND 10

PLATE 9

Fig. 1. Seedling leaves of Wilma inoculated with race 6 (left) and race 6*x* (right).

Fig. 2. Spores of race 3 after incubation at 15° C. (*a*) and 20° C. (*b*) for 24 hr.

PLATE 10

Fig. 1. Leaves of mature plants of Wilma inoculated with race 3 (left), race 8 (centre) and race 6 (right).

Fig. 2. Leaves of mature plants of Jubilegem inoculated with race 3 (left), race 8 (centre) and race 6 (right).

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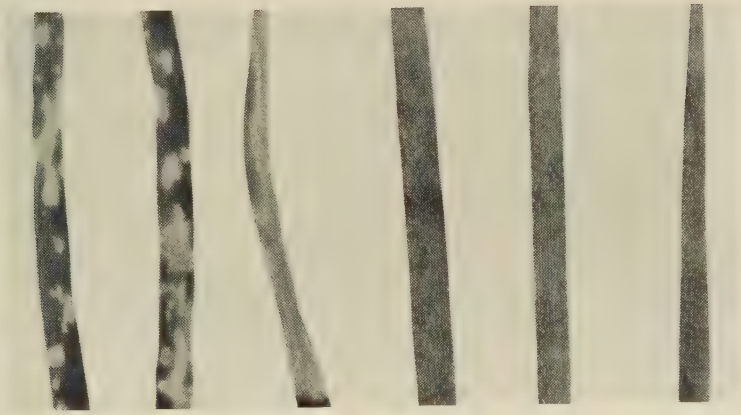

Fig. 1

Fig. 2*a*

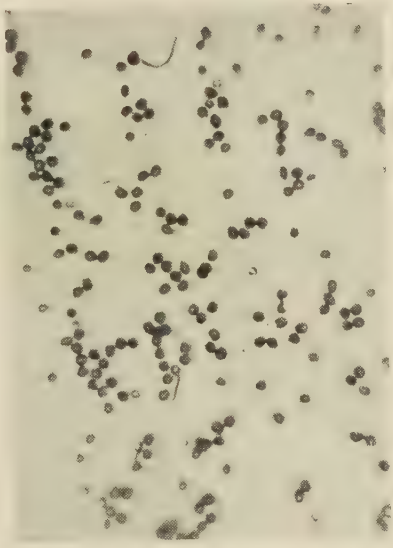

Fig. 2*b*

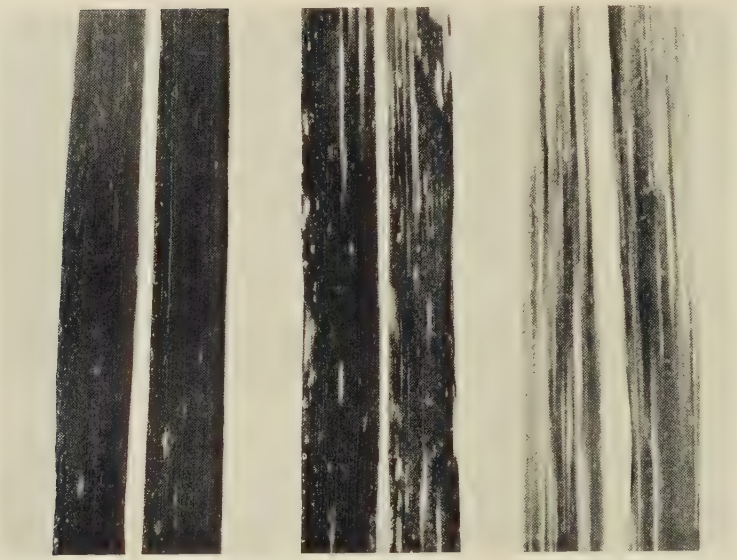

Fig. 1

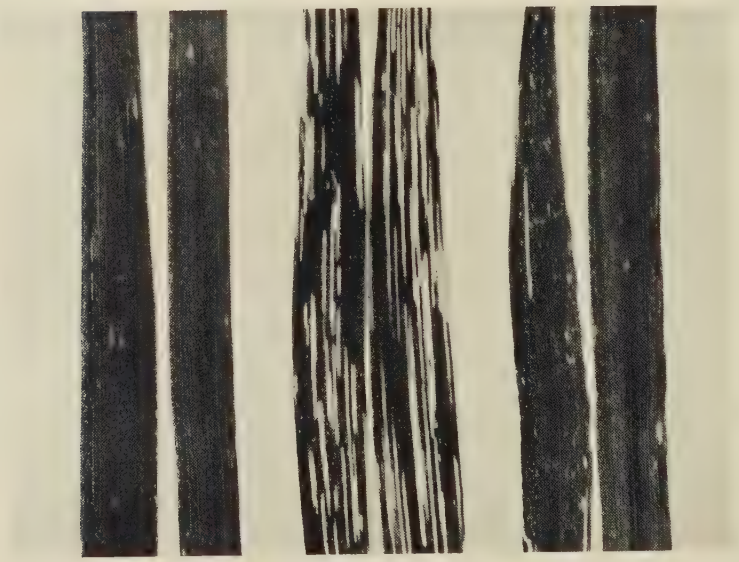

Fig. 2

MANNERS—*Studies on the physiologic specialization of yellow rust*

SOME EFFECTS OF HOST-PLANT NUTRITION ON THE MULTIPLICATION OF VIRUSES

By F. C. BAWDEN and B. KASSANIS
Rothamsted Experimental Station, Harpenden, Herts

The amounts of tobacco mosaic virus present in systemically infected tobacco plants varied greatly with the mineral nutrition of the plants and were related to the effects on plant growth. With plants in soil, supplements of phosphorus produced the greatest increases in plant size, in virus concentration of expressed sap, and in total virus per plant; nitrogen increased plant size only when phosphorus was also added, and only then increased virus concentration and total virus per plant. Combined supplements of phosphorus and nitrogen doubled the virus concentration of sap and increased the total virus per plant by factors up to forty. Potassium slightly reduced the virus concentration of sap, though it usually increased plant size and total virus per plant. From all plants, only about one-third of the virus contained in leaves was present in sap. Virus production seemed to occur at the expense of normal plant proteins, and the ratio of virus to other nitrogenous materials was highest in plants receiving a supplement of phosphorus but not of nitrogen.

The effects of host nutrition on the production of virus in inoculated leaves resembled those in systemically infected leaves, but were more variable.

No evidence was obtained, with plants grown in soil or sand, that host nutrition had any consistent effect on the intrinsic infectivity of tobacco mosaic virus.

The concentration of virus in sap from potato plants systemically infected with two strains of potato virus *X* was not consistently affected by fertilizers; the chief effect of host nutrition on virus production was indirect by altering plant size.

Viruses appear to be obligate parasites and as such it might be expected that their ability to multiply will be affected by changes in host-plant metabolism produced by varying nutrition. Surprisingly few experiments have been made on the subject, and published work is restricted to the manner in which changes in supply of nitrogen affect tobacco mosaic virus. Even so, the results are confused and conflicting. Rischkov & Smirnova (1939) stated that, in tomato, the virus reached the same concentration in nitrogen-deficient plants as in those not deficient, whereas Spencer (1939) stated that, in tobacco, virus concentration depended directly on the amount of nitrogen supplied to the host. He concluded that the concentration of virus in sap from plants receiving abundant nitrogen was eighty times as great as that in sap from nitrogen-deficient plants and that increasing nitrogen still increased virus concentration even when the amounts supplied were sufficient to inhibit plant growth. Spencer estimated virus concentration from measurements of relative infectivity, a method he later (1941 *b*) concluded was justified, for he stated that the results obtained from it agreed closely with the amounts of virus that could be isolated from different lots of sap by ultracentrifugation.

In other papers, however, Spencer (1941 *a*, *c*, 1942) described results that seem to

conflict with this conclusion, and he claimed that nitrogen affects the intrinsic infectivity of the virus as well as the amount produced. The addition of nitrogen to plants recently infected was said to increase both the rate of virus multiplication and the infectivity per unit weight of the virus produced, and the interruption of the nitrogen supply to systemically infected plants was stated to retard or inhibit virus multiplication and to lead to a reduction in infectivity per unit weight of virus. The effects of increasing nitrogen on virus concentration described in the later papers were much smaller than those originally claimed, though comparisons are difficult because sometimes yields were recorded as mg. per ml. of sap and sometimes as mg. per leaf. Spencer (1941 *a*, *b*) concluded that the virus depends for multiplication on simple forms of nitrogen and cannot utilize the normal plant proteins, a conclusion at variance with other observations (Martin, Balls & McKinney, 1939; Takahashi, 1941; Woods & Dubuy, 1941), which suggest that it multiplies at the expense of normal plant proteins and that, when nitrogen is deficient, it increases while these proteins are autolysing.

The experiments now described were made to determine in what manner wide variations in the amounts of nitrogen, phosphorus and potassium, and in the relative proportions of the three nutrients, supplied to host plants affected the multiplication of tobacco mosaic virus and potato virus *X*, and the effects of such variations on the intrinsic infectivity of tobacco mosaic virus.

Materials and methods

The viruses and hosts used were tobacco mosaic virus in tobacco (*Nicotiana tabacum*, var. White Burley) and potato virus *X* in potato (*Solanum tuberosum*, vars. Majestic and Doon Star). The experimental design and methods of growing the plants and adding fertilizers were similar to those used in experiments on the effects of nutrition on susceptibility to infection (Bawden & Kassanis, 1950).

In most experiments with tobacco, seedlings were transplanted at the four-leaf stage to unglazed pots containing 1 kg. of a mixture of 50% infertile soil, and 25% respectively of sand and peat, to which the fertilizers had previously been added as solids. The amount added for each plant receiving a supplement of nitrogen was 2·82 g. $(NH_4)_2SO_4$, of phosphorus 0·76 g. $Ca(H_2PO_4)_2$, $2H_2O$, and of potassium 1·12 g. K_2SO_4. There were five plants in each of eight different treatments; no supplement added (1); nitrogen only (*n*); phosphorus only (*p*); nitrogen and phosphorus (*np*); potassium only (*k*); nitrogen and potassium (*nk*); phosphorus and potassium (*pk*); nitrogen, phosphorus and potassium (*npk*). Some experiments were also made with tobacco plants growing in sand, and in these either nitrogen or phosphorus was varied while all other nutrients were kept constant. The nutrients were added in solution, 100 ml. of the solutions described by Spencer (1937) being given to each plant thrice weekly.

The inoculum was a purified preparation of tobacco mosaic virus which, unless otherwise stated, was used at 0·1 mg./ml. When multiplication was being studied in

inoculated leaves, the plants were inoculated 2–4 months after potting, when they had started to shoot and those receiving a supplement of phosphorus were about 6 in. high. Four leaves occupying equivalent positions on each plant were rubbed over their whole upper surfaces with the forefinger wet with inoculum, and the plants were harvested 10–14 days later. When multiplication was being studied in systemically infected leaves, plants were inoculated while still in the rosette stage, from 1 to 2 months after potting. The two lowest leaves on each plant were inoculated, and the plants were harvested a month or more later.

At harvest, the plants were cut at soil level and weighed individually to record the effects of the treatments on plant growth. The leaves were then picked, and those from plants receiving similar treatments were pooled and weighed. In experiments on systemic multiplication all the leaves were picked, and in others only those that were inoculated. The leaves were macerated by passage through a domestic meat mincer, when the sap was expressed by squeezing through muslin and its volume measured. A sample from each treatment was placed at $-7°$ C. and kept frozen until used for infective tests. The relative virus contents of the different lots of sap were then estimated serologically, to act as check on the recovery achieved by the purification technique employed. Samples were heated to 60° C. and centrifuged; this not only gives an efficient clarification, but also brings the virus particles into comparable states of aggregation so that precipitin tests can be used quantitatively with greater reliability (Bawden & Pirie, 1945). The precipitin titres of the different lots of clarified sap were then found by adding 1 ml. at various dilutions to each of a series of tubes containing 1 ml. of tobacco mosaic virus antiserum at a dilution of 1/200, the titre being taken as the highest dilution at which a precipitate visible to the eye was produced after 2 hr. in a water-bath at 50° C. The differences in virus content indicated by these tests were always reflected in comparable differences between the yields of virus that were isolated from the different lots of sap. The isolation and weighing of purified virus, however, was more sensitive, and showed differences too small to be detected with certainty by precipitin tests. The values obtained refer, of course, only to virus that remains in solution after clarifying the sap. We have no evidence that the clarification causes appreciable losses, or that the losses vary with different lots of sap, but if they do, then our figures will not necessarily reflect the original differences.

For the isolation of the virus, 10 ml. samples of sap were used when possible, but with some of the plants that grew poorly smaller ones had to be used. After clarifying the sap by heating to 60° C. and centrifuging, the virus was isolated from the supernatant fluids by the method described by Bawden & Pirie (1943), involving repeated precipitation with ammonium sulphate and acid. The yield was determined by drying and weighing a sample of the final salt-free product. The precipitin titres of the purified preparations were tested and the agreement between these and those obtained on the clarified sap suggested that the recovery of virus was substantially complete. Bawden & Pirie (1937) showed that the ratio of phosphorus to carbo-

hydrate is a useful guide to the purity of preparations of tobacco mosaic virus; analyses made on our purified products all fell within the ranges phosphorus 0·47–0·52% and carbohydrate 2·0–2·5%, indicating that there was no gross contamination.

Comparisons of the infectivity of the virus obtained from plants receiving different fertilizers were made by the local-lesion method, using *Nicotiana glutinosa* as a host. For each comparison of eight samples, eight plants were used each with eight leaves, and half-leaves were inoculated according to two superimposed Latin square designs, so that each sample was inoculated to sixteen half-leaves. The samples were compared at two dilutions, the purified preparations being used at 10 and 1 mg./l. and the samples of sap at dilutions corresponding to these virus contents as calculated from the yields of virus isolated from them. For example, if the amount of virus isolated was 1·5 mg./ml. of sap, the tests were made with the sap diluted 1/150 and 1/1500. Purification usually reduced the numbers of lesions produced by the virus, that is, at equivalent virus contents sap was more infective than the purified virus, but it affected all samples similarly. Hence the relative infectivities of virus from plants receiving different treatments were unaffected by purification and only the results obtained with sap are given in the tables.

The standard errors of local-lesion counts increase linearly with increasing means, so that customary tests for statistical significance cannot be applied to them directly (Kleczkowski, 1949). Before analysing the results statistically, therefore, the counts were increased by 1 and transformed into logarithms.

Experiments with potato virus *X* were made with potato plants in their second year of infection, and all with sand-soil-peat mixtures to which the fertilizers were added in solid form at the time infected sets were planted. The sets were pieces of a uniform size, each carrying a single 'eye', from infected tubers, and were planted in pots containing 2 kg. of the potting medium, and the fertilizer supplements were twice as large as those used for tobacco plants. The virus content of the haulms was estimated serologically, expressed sap being heated to 60° C., centrifuged, and then titrated against antiserum to potato virus *X* to determine its precipitation end-point.

Results

Effect on plant growth

The different fertilizer treatments affected the appearance and growth of the plants in much the same manner as already described from experiments on the effects of nutrition on susceptibility to infection (Bawden & Kassanis, 1950). With tobacco plants grown in soil, phosphorus was the most effective element in increasing plant size, nitrogen usually did so only in the presence of phosphorus, and potassium had little effect. These differences are shown in the various tables by the fresh weights of the plants at harvest. Plants used in tests on local multiplication of tobacco mosaic virus did not differ appreciably from uninfected ones receiving the same nutrients. The systemically infected plants were all smaller and paler than their

uninfected counterparts, and there was no single element or combination with any striking effects on the disease, but symptoms were usually most severe on the smallest plants. These also suffered a proportionally greater loss of yield; 4 weeks or so after infection, the smallest plants (treatments (1), *n* and *k*) usually weighed little more than half as much as their uninfected controls, whereas the largest plants (treatments *np* and *npk*) usually weighed about three-quarters as much as their uninfected controls. Because of the large differences in size produced by the different fertilizers, however, the greatest total loss of weight occasioned by infection occurred with the largest plants.

Analysis of sap and fibre from the leaves in which local multiplication of the virus was being studied showed that the distribution of nitrogen, phosphorus and protease did not differ appreciably from that in uninfected leaves. By contrast, systemic infection produced large changes; in addition to the reduction in fresh weight already mentioned, it reduced the total dry matter, the proportion of dry matter to fresh weight, the total phosphorus and the protease activity in sap per g. of protein; it had no effect on the total nitrogen, but increased the proportion of nitrogen and phosphorus to other dry matter, the nitrogen content of sap and the protease activity per g. of dry weight (Holden & Tracey, 1948).

Effect on multiplication of tobacco mosaic virus

In experiments with systemically infected tobacco plants grown in soil considerable and consistent effects of fertilizers on virus multiplication were obtained. The results of two typical experiments are shown in Table 1, from which the most immediately obvious effect is that of phosphorus in increasing both plant growth and the concentration of virus in the sap. Analysis for the main effects and interactions (Yates, 1937) shows that phosphorus and nitrogen stimulated both plant growth and virus concentration. Phosphorus had a large positive effect on both, nitrogen a much smaller positive effect, and there was also a positive nitrogen-phosphorus interaction. In the conditions of these experiments, nitrogen increased plant growth only in the presence of phosphorus, and similarly it increased virus concentration in the sap only in the presence of phosphorus. In both experiments shown in Table 1, the lowest virus concentration occurred with treatment *n* and the highest with *np*. The agreement between plant growth and virus concentration, however, was not complete, for whereas potassium had a positive effect on plant growth it had a slight negative effect on virus concentration.

A complete picture of the extent to which conditions favour virus multiplication is not provided by measurements of virus concentration in expressed sap, but only by measurements of the total amounts of virus contained in the plants. We have not attempted to measure these with great accuracy, but values that are thought to express the relative virus contents are recorded in Table 1 under the column headed 'total virus in sap'. These values are the products of virus concentration in the sap and the volume of sap obtained by macerating and squeezing the leaves from the

Table 1. *The effects of fertilizers on the multiplication of tobacco mosaic virus in systemically infected tobacco plants grown in soil*

Treatment	Mean wet wt. of plants (g.)	Virus concentration in sap (g./l.)	Total virus in sap (mg.)	Infectivity of virus at 10 mg./l.	Infectivity of virus at 1 mg./l.
Exp. 1 (plants potted 21 May, infected 11 June, harvested 12 July)					
(1)	22	2·92	39	1·00*	0·69*
n	32	2·60	49	1·12	0·68
p	39	3·26	70	1·19	0·64
np	91	4·78	191	1·09	0·27
k	28	2·82	45	1·00	0·78
nk	42	2·22	54	1·03	0·45
pk	41	3·24	73	0·92	0·28
npk	112	4·52	226	1·06	0·40
Significant difference:					
5 % level	8·9	—	—	0·37	0·34
1 % level	12·0	—	—	0·48	0·45
Exp. 2 (plants potted 31 July, infected 20 August, harvested 24 September)					
(1)	9	2·32	13	1·75	1·22
n	4	2·08	4	1·68	0·91
p	22	4·26	54	1·71	1·21
np	52	4·98	126	1·61	1·10
k	8	2·38	11	1·71	1·21
nk	12	1·84	13	1·79	1·20
pk	28	3·00	43	1·70	1·10
npk	72	4·42	172	1·81	1·12
Significant difference:					
5 % level	7·5	—	—	0·23	0·31
1 % level	10·1	—	—	0·30	0·41

* Numbers of lesions (x) expressed as $\log_{10}(x+1)$.

differently fertilized plants. The main source of error in these comparisons is the incomplete recovery of sap; a larger proportion of the total sap will be lost from small than from large plants, so the effect of increasing plant size will tend to exaggerate the stimulating effect of increasing size on virus production. When the results are expressed in this manner, virus production is directly related with plant growth, for the slight reduction in virus concentration produced by supplements of potassium are more than compensated for by the increase in the volume of sap obtained from the larger plants. Except for this effect of potassium, concentration of virus is related to plant growth, so that expressing the results as total virus also enhances the effects of varying nutrition on virus multiplication. Thus, of the experiments recorded in Table 1, in the first, there is less than a factor of two between the smallest and largest virus concentrations but a factor of more than five between the smallest and largest total weights of virus; in the second experiment, the increase is much greater, the largest difference between virus concentrations being a factor of between two and three and that between total virus contents being about forty. Hence, although nutrition of the host has considerable effects on the

amount of virus produced per unit weight of host tissue, its main effect on virus multiplication is indirect by altering host size and the amount of tissue in which multiplication can occur.

The weights given for the total virus in the sap do not show the total virus content of the plant. After leaves have been macerated and the sap expressed, the residual fibre still contains much virus, which is not released by extraction with water but is, in part, by fine grinding and, more completely, by incubation with the enzyme mixture contained in snails' stomachs (Bawden & Pirie, 1945, 1946). Samples of the leaf fibre from all plants used in the fertilizer experiments were assayed for their virus contents, by determining the precipitin titres of extracts of fibre ground in a triple-roller mill and of extracts made by repeated incubations with snail enzymes. These estimates were occasionally checked by purifying and weighing the virus released from the fibre by fine grinding to ensure that precipitin titres were adequately indicating virus contents. From all leaf samples, the amount of virus released from the ground fibre was approximately the same as that obtained in the sap, whereas incubation with snail enzymes released about twice this amount. Thus, regardless of the total virus content of the leaves, a constant proportion, about one-third, is released in the sap that can be squeezed from macerated leaves, and the amounts obtained in different lots of sap can be taken as indicating the relative virus contents of different leaves.

The results of experiments on the amounts of virus produced in inoculated leaves were more variable than those with systemically infected plants, which is only to be expected as there are more uncontrolled variables. In systemically infected plants, all susceptible cells are probably infected, and differences in virus concentration of sap probably reflect the manner in which changes in host nutrition affect the ability of the cells to support virus multiplication. The concentration in sap from inoculated leaves, however, will also be affected by the number of initial entry points and the rate at which virus diffuses from the first infected cells to their neighbours. Despite these complicating factors, reasonably similar effects on virus concentration by fertilizers were obtained in both kinds of experiment. The results of two experiments on local multiplication of tobacco mosaic virus in plants grown in soil are given in Table 2. Analysis for main effects and interactions shows that, except for potassium, additions that increased plant growth also increased virus concentration. In both experiments phosphorus had a large positive effect on both growth and virus concentration; in one nitrogen increased both growth and virus concentration and in the other it decreased both. Responses to potassium, however, were variable; in Exp. 1, the main effect and interactions were all positive on growth, but negative on virus concentration, whereas in Exp. 2, there was little effect on growth, but a positive main effect on virus concentration.

Table 3 shows the effects of varying the virus concentration of the inoculum on the concentration of virus obtained in sap from the rubbed leaves. Two different fertilizer treatments were used, namely, no additions to the soil-sand-peat mixture

Table 2. *The effects of fertilizers on the multiplication of tobacco mosaic virus in inoculated leaves of tobacco plants grown in soil*

Treatment	Mean wet wt. of plants (g.)	Virus concentration in sap (g./l.)	Infectivity of virus at 10 mg./l.	Infectivity of virus at 1 mg./l.
Exp. 1 (plants potted 31 October, infected 12 February, harvested 22 February)				
(1)	20	0·15	1·50*	1·24*
n	15	0·20	1·28	0·89
p	49	0·37	1·57	1·23
np	76	1·15	1·66	1·46
k	21	0·13	1·51	1·47
nk	13	0·37	1·72	1·49
pk	51	0·13	1·45	1·42
npk	106	0·32	1·82	1·56
Significant difference:				
5 % level	12·6	—	0·40	0·34
1 % level	17·0	—	0·54	0·46
Exp. 2 (plants potted 1 October, infected 17 December, harvested 30 December)				
(1)	16	0·15	1·34	0·82
n	4	0·18	1·32	0·64
p	36	0·64	1·69	1·41
np	36	0·55	1·73	1·36
k	12	0·26	1·71	0·89
nk	6	0·21	1·69	0·85
pk	41	1·06	1·84	1·07
npk	33	0·78	1·84	1·27
Significant difference:				
5 % level	7·2	—	0·42	0·57
1 % level	9·7	—	0·57	0·76

* Numbers of lesions (x) expressed as $\log_{10}(x+1)$.

Table 3. *Effect of concentration of inoculum on the local multiplication of tobacco mosaic virus receiving different fertilizers*

(Plants potted in soil 31 December, inoculated 11 March, harvested 25 March)

Fertilizer treatment	Virus in inoculum (g./l.)	Mean wet wt. of plants (g.)	Virus concentration in sap (g./l.)	Infectivity of virus at 10 mg./l.	Infectivity of virus at 1 mg./l.
None	0·08	9	0·10	1·61*	1·13*
	0·4	10	0·32	1·70	1·55
	2·0	8	0·46	1·60	1·04
npk	0·08	43	0·70	1·83	1·50
	0·4	39	0·73	1·76	1·58
	2·0	36	1·19	1·75	1·65
Difference significant at 5 % probability level				0·20	0·74

* Numbers of lesions (x) expressed as $\log_{10}(x+1)$.

and the full *npk* treatment. The different inocula were each applied to five plants from the two treatments, four equivalent leaves on each plant being rubbed over their

whole upper surfaces. The virus concentration in sap from all the *npk* plants exceeded that in any of the others, but the greatest difference between the differently fertilized plants was with the most dilute inoculum. Differences between the number of initial infection sites may therefore play some part in determining differences between the virus concentration achieved in the inoculated leaves. It is probably only a small part, however, for the differences between amounts of virus extracted from the inoculated leaves are much greater than those between the numbers of local lesions produced per unit leaf area on differently fertilized plants (Bawden & Kassanis, 1950); also, with inoculum twenty-five times as concentrated as that used for the *npk* plants, the concentration of virus in sap from the unfertilized plants still failed to reach that in the *npk* plants. It seems, then, that in inoculated leaves, as in those that are systemically infected, virus multiplication proceeds more rapidly and extensively when host nutrition is such as to produce a vigorous plant than when growth is slow.

None of the experiments with plants grown in soil gave any results to support Spencer's (1939) statement that nitrogen specifically stimulates virus multiplication and that this effect is independent of effect on plant growth. Most of his experiments were made with plants in sand supplied with nutrient solutions, and as he also stated that this treatment produced plants with a higher virus content than fertile soil, even though the plants grew better in the soil, it seemed that the discrepancy might be caused by the difference in technique. However, experiments we have made in sand culture gave plants with lower virus concentrations than found with vigorous plants in soil, and the effects of fertilizers on virus concentration were also smaller. Table 4

TABLE 4. *The effect of varying nitrogen and phosphorus on the multiplication of tobacco mosaic virus in tobacco plants grown in sand*

	Mean wet wt. of plants	Virus concentration in sap	Total virus in sap	Infectivity of virus at	
N (mg./day)	(g.)	(g./l.)	(mg.)	10 mg./l.	1 mg./l.
		Varying nitrogen			
0·4	10	1·86	9·1	1·83*	1·34*
8	23	1·83	26·7	1·57	1·47
80	2·3	2·33	2·33	1·75	1·14
	Difference significant at 5 % probability level			0·26	0·40
		Varying phosphorus			
P (mg./day)					
0·4	8	1·91	8·0	1·58	1·02
8	17	2·18	20·5	1·59	1·04
60	1	3·11	1·9	1·43	1·12
	Difference significant at 5 % probability level			0·44	0·44

(Nutrients added in solution thrice weekly, all being kept constant except for variations in either nitrogen or phosphorus.)

* Numbers of lesions (x) expressed as $\log_{10}(x+1)$.

gives the results of two such experiments; the three levels of nitrogen and phosphorus used were such that the medium level stimulated growth and the high level reduced it. The differences in virus concentration were the largest obtained in such experiments, and there is a suggestion that levels of phosphorus and nitrogen, and particularly phosphorus, may increase virus concentration even when they are inhibiting plant growth. The increases were too slight to compensate for the reduction of host tissues, and in these experiments, as in those with soil, maximum virus production occurred in the largest plants.

Effect of host nutrition on infectivity of tobacco mosaic virus

Since Spencer (1941*a*, 1942) stated that increasing the supply of nitrogen to host plants increased the infectivity per unit weight of the tobacco mosaic virus produced, Bawden & Pirie (1945) have fractionated the virus in sap by differential centrifugation into preparations that differ widely in particle size and infectivity per unit weight, the most rapidly sedimenting particles being, weight for weight, more than a hundred times as infective as the most slowly sedimenting ones. It seemed that host nutrition might be altering the ratios at which particles with different characters occur in sap, and we had intended to test this hypothesis by comparing the behaviour of sap from plants receiving different fertilizer treatments when ultracentrifuged. This has proved unnecessary, because we have been unable to demonstrate any consistent reproducible effect of host nutrition on the intrinsic infectivity of the virus present in sap. The results of infectivity tests comparing virus from the differently fertilized plants at the same concentrations are recorded in Tables 1–4. In most of the comparisons one or more of the inocula usually produced a number of lesions that differed from other numbers sufficiently to be statistically significant, but there is no one element that consistently increased or decreased infectivity. From the mean of all such experiments, the lowest infectivity was given with virus from treatments (1) and *n*, but these treatments also gave the smallest yields of purified virus and so offered the largest opportunities for error in determining virus concentration; also, if the method of purification used gives a constant yield of normal plant materials, the ratio of these to virus will be highest in these treatments.

Effect on the multiplication of potato virus X

Quantitative isolations of the type used for determining the amounts of tobacco mosaic virus present in sap are impractical with potato virus *X*, which tends to become insoluble when attempts are made to purify it (Bawden & Kleczkowski, 1948). Estimates of the relative virus concentrations in sap from plants receiving different fertilizer treatments were made from measurements of precipitin titres, a less sensitive method than isolation and weighing, but sufficiently accurate to show differences of a factor of two unequivocally and smaller differences with less certainty. Two precipitin tests were made, one in which clarified sap was diluted serially by a factor of two, to obtain the approximate end-point, and another in which the titre was

determined more accurately by testing a series of dilutions around the end-point, the dilutions varying by only small factors. Table 5 shows the results of three experiments, no. 1 done with Majestic infected with an avirulent strain that caused

TABLE 5. *The effect of fertilizers on the concentration of potato virus X in potato plants grown in soil*

	Mean wet wt. of plants (g.)			Relative virus concentration in sap*		
Treatment	Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp. 3
(1)	45	40	54	160	800	1000
n	22	83	87	80	600	1000
p	41	33	73	320	600	1400
np	34	142	144	320	800	2000
k	43	33	73	40	600	1000
nk	52	101	99	40	600	1500
pk	53	49	109	320	800	1200
npk	78	200	156	80	600	2000

Exp. 1, Majestic with an avirulent strain, tubers potted 2 April, plants harvested 5 June; Exp. 2, Majestic with X^Y, tubers potted 14 May, plants harvested 21 July; Exp. 3, Doon Star with X^Y, tubers potted 19 June, plants harvested 19 August.

* Virus concentration expressed as reciprocal of precipitin titre.

a barely perceptible mottle and nos. 2 and 3, respectively, with Majestic and Doon Star infected with strain X^Y (Bawden & Kleczkowski, 1948), which causes a bright yellow interveinal mosaic. There are no obviously reproducible effects of host nutrition on the concentration of virus X in the sap, and under all nutritional conditions the concentration of X^Y considerably exceeded that of the avirulent strain. Fewer and smaller differences in cencentration occurred with X^Y, despite its greater multiplication, than with the other strain, suggesting that with neither was multiplication being limited by the scarcity of mineral nutrients. There are similarities between the results of these experiments and those obtained with tobacco plants systemically infected with tobacco mosaic virus; in Exp. 1, for example, phosphorus stimulated both plant growth and virus concentration and potassium reduced virus concentration but increased growth. There are others, but none is worth stressing, for an equal number of dissimilarities are also evident. In other conditions, or in other hosts, it may be that mineral nutrition has larger effects on the concentration of virus X, but in these experiments the only result worth stressing is the extent to which total virus multiplication in systemically infected plants was increased by conditions that increased plant size.

DISCUSSION

Our results gave no evidence to substantiate the claims made by Spencer that both the multiplication and the intrinsic infectivity of tobacco mosaic virus are specifically affected by the amount of nitrogen supplied to host plants. No definite interpretation of these discrepancies is possible, but it may be that we used a strain of tobacco

mosaic virus that reacts differently to changes in nitrogen supply. Similarly, we made our infectivity tests on *Nicotiana glutinosa*, whereas Spencer used Golden Cluster beans, and it may be that nitrogen affects infectivity towards one host and not towards another. However, there are also considerable discrepancies between the claims made at different times by Spencer for the effects of nitrogen on the virus, and these suggest that the techniques used were insufficiently accurate to justify the conclusions drawn from the results. For example, the largest effect of nitrogen on virus concentration in the sap, an eighty-fold increase, was described when virus concentration was being estimated solely from differences in lesion counts (Spencer, 1939), and the increases described when the virus was isolated by ultracentrifugation (1941 *a*, *c*, 1942) were much smaller, usually less than factors of two. These are of the same order as we have found, though in our work the increases in concentration were usually correlated with increases in plant growth and were more usually produced in response to supplements of phosphorus than to nitrogen. In measuring the relative virus concentrations in different lots of sap by isolating and weighing the virus, comparisons are relevant only if the method used recovers all the virus from each sample and nothing but virus. Spencer provided no evidence on either of these points, for he made no tests on the initial sap or analyses on the final product. His method was simply to ultracentrifuge clarified sap, clarify the resuspended pellet by low-speed centrifugation, and then repeat the process a second time. The amount of nitrogen in the final supernatant fluid was determined and used to calculate the quantity of 'virus protein', a term he defined as 'the proteinaceous sediment obtained by repeated ultracentrifugation', and which Spencer (1942) described by saying 'Although this material is practically homogeneous, it is not necessarily crystalline.' The basis of the claim for homogeneity is not stated, and there would seem little reason to assume that all the preparations consisted exclusively of virus. The conclusion that nitrogen increased the specific infectivity of the virus was based on differences between lesion counts that were rarely greater than factors of two, and without either estimates of error or information on the purity of the products being compared, the significance of these differences is a matter of speculation.

Experiments on the mineral nutrition of host plants are hardly to be expected to provide information on the mechanism of virus multiplication, but Spencer (1941 *a*) suggested that the process utilizes simple forms of nitrogen rather than nitrogen already synthesized into normal proteins, and that the normal requirements of leaves need to be satisfied before the virus multiplies. This suggestion was first made when he concluded that the virus did not multiply in nitrogen-deficient leaves, but was reaffirmed subsequently (Spencer, 1942) even though, like other workers (Martin *et al.* 1939; Takahashi, 1941; Woods & Dubuy, 1941), he then found that it did multiply in nitrogen-deficient leaves. The analyses made by Holden & Tracey (1948) on the plants from our experiments certainly do not suggest that the multiplication of tobacco mosaic virus depends on an external supply of simple forms of nitrogen in excess of that needed for producing normal plant proteins. Rather they suggest that

the virus is produced at the expense of normal proteins, for the ratio of virus to other nitrogenous constituents is greatest in nitrogen-deficient plants. Expressed as a mean of all treatments, about one-third of the total nitrogen occurred in the virus, but the range was from 10 to 60%, depending on the fertilizer treatment, and in one experiment reached 80% with plants that received a supplement of phosphorus but not of nitrogen. When correcting a gross deficiency, extra nitrogen may increase the virus content per g. of leaf, but other nitrogen-containing materials are increased still more, and the ratio of virus to total nitrogen is decreased. With both nitrogen and phosphorus, effects on virus multiplication mainly reflected responses in plant growth, nutrition that stimulated vigorous host plants also increased virus production, both per unit weight of leaf and, most strikingly, per plant. There is a suggestion that this may not be true with potassium, which often stimulated plant growth but gave less extractable virus per unit weight of leaf, but without further knowledge it would be premature to assume that potassium has any specific action in inhibiting the multiplication of tobacco mosaic virus.

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VIRUS DISEASES OF CACAO IN WEST AFRICA

V. ALTERNATIVE HOST PLANTS

By A. F. POSNETTE*, N. F. ROBERTSON† and J. McA. TODD

West African Cacao Research Institute, Tafo, Gold Coast

(With Plates 11 and 12)

Of the tested plants which are indigenous to West Africa, three species in the Bombacaceae and four in the Sterculiaceae are susceptible to one or more of four viruses from cacao, three occurring in the Gold Coast and one in Nigeria. These species are less affected than cacao by the viruses; some show transient leaf symptoms and others are symptomless carriers. The development of spines on the stems of *Ceiba pentandra* seedlings is suppressed by infection with virus 1A.

In general, the indigenous species are more difficult to infect than cacao, and mealybugs do not become infective as readily when feeding on them as when feeding on infected cacao. The availability of the viruses to vectors seems to be correlated with severity of symptoms, and transmission from infected plants to cacao becomes less frequent with increasing duration of infection.

Ceiba pentandra trees were found naturally infected in the Gold Coast and Nigeria. In the Western Province of the Gold Coast, *Cola chlamydantha* trees in cacao farms and forests were naturally infected with viruses apparently identical with those causing swollen shoot of cacao there.

There is little doubt that *C. chlamydantha* trees are an important source of virus for cacao trees. Whenever possible these and other alternative hosts growing near to cacao should be destroyed.

Introduction

As swollen shoot was the first disease of cacao shown to be caused by a virus, it seemed probable that it had spread to cacao from plants indigenous to West Africa. To test this possibility, work on the host range of virus 1A (Posnette, 1947) was started in 1940, by grafting infected cacao plants to seedlings of indigenous species and afterwards grafting the latter to healthy cacao plants. This technique gave transmissions to and from seedlings of *Theobroma bicolor* Humb. & Bonpl. (introduced from Nicaragua and the only available species closely related to *T. cacao* L.), but no virus was passed through any indigenous plant to cacao. As the graft unions were only temporary, this failure did not prove that the indigenous plants were immune, but after an unsuccessful attempt to use the local dodder (*Cuscuta chinensis* Lav.) as a vector, it was clear that these investigations must await a reliable technique for transmission by insects.

The host range of virus 1A

Work on the alternative host plants of virus 1A was resumed in 1946 when transmission by mealybugs was well established. Twelve seedlings of each of five

* Now at East Malling Research Station, Kent.
† Now at the Botany School, Cambridge University.

suspected indigenous species were grown in pots in insect-proof houses and colonized with infective mealybugs (*Pseudococcus njalensis* Laing.), which were either collected from infected cacao trees in the field and transferred direct to the test plants, or reared on infected cacao seedlings in the greenhouse. Fifty infective insects were transferred to each indigenous plant and ten to each of five cacao seedlings as a check on infectivity. The indigenous plants were colonized three times, so that each received 150 vectors.

From 3 to 14 months after the third colonization, each indigenous plant was infested with virus-free mealybugs, which were transferred to cacao seedlings after a few days' feeding. The results showed that three seedlings of *Ceiba pentandra* Gaertn. were systemically infected, and that the seedlings of *Sterculia rhinopetala* K. Schum. and of *S. tragacantha* Lindl. were probably not: single transmissions from *Bombax buonopozense* P. Beauv. and *Triplochiton scleroxylon* K. Schum. were regarded as inconclusive evidence of systemic infection.

A second experiment, which gave results included in Table 1, proved that *Ceiba pentandra* and *Bombax buonopozense* can carry the cacao virus 1 A and suggested that *Triplochiton scleroxylon* probably cannot.

By this time, vector experiments with cacao had demonstrated the advantages of using seeds or very young cacao seedlings as test plants (Posnette & Strickland, 1948). This method was therefore adopted for testing indigenous plants as virus hosts, using cacao beans as indicator plants in testing for virus content after the alternative host seedlings had been subjected to infective mealybugs. Some common species in the Tiliales were first tested, followed by unrelated species frequently associated with swollen shoot outbreaks.

The results given in Table 1 show that, from among the plants tested, the host range of virus 1 A is restricted to plants in the Bombacaceae and Sterculiaceae. Members of all genera of the Bombacaceae represented in the West African flora can become systemically infected. *Adansonia digitata* Linn. was the most easily infected, and also gave a high percentage of transmissions to cacao; *Bombax buonopozense* and *Ceiba pentandra* were more resistant, and only a low percentage of transmissions to cacao was usually obtained.

Symptoms

Infected seedlings of *Adansonia digitata* were severely stunted and developed conspicuous leaf symptoms which persisted in successive growth flushes. *Bombax buonopozense* seedlings were only slightly affected and symptoms were confined to vein-clearing in one or two leaves. The low rate (with one exception) of virus transmission to cacao suggested that virus content might usually be lower than in other host plants. The stems of infected *Ceiba pentandra* seedlings developed raised areas which became necrotic; at first a few leaves showed conspicuous vein banding, but later the plants became symptomless except for the necrosis and partial suppression of spines on the stem (Pl. 11, fig. 1). The reduction in severity of symptoms

TABLE 1. *Experiments with cacao virus 1A*

Plant species tested	Infection rate*		Back transmission tests to cacao		
	Cacao controls	Alternative host	From infected plants	From healthy plants	Insects per plant
MYRISTICACEAE					
Pycnanthus kombo Warb.	15/27	0/4	—	0/30	20
MALVACEAE					
Gossypium hirsutum Linn.	6/13	0/5	—	0/138	20
TILIACEAE					
Desplatzia lutea A. Chev.	5/5	0/5	—	0/97	20
STERCULIACEAE					
Cola chlamydantha K. Schum.	5/10	0/5	—	0/319	10
C. chlamydantha K. Schum.	12/18	0/5	—	0/279	10
C. cordifolia R.Br.	15/25	4/16	7/63	0/31	10
C. cordifolia R.Br.	0/15	3/4†	—	0/94	10
C. nitida A. Chev.	5/6	0/5	—	0/91	10
C. togoensis Engl. & Krause	11/15	0/19	—	0/110	20
Erythropsis barteri K. Schum.	5/10	1/3	7/8	0/9	20
Sterculia rhinopetala K. Schum.	8/22	0/11	—	0/203	10
S. tragacantha Lindl.	15/26	0/11	—	0/278	10
S. tragacantha Lindl.	2/4	0/10	—	0/97	20
Triplochiton scleroxylon K. Schum.	3/7	0/10	—	0/175	10–30
BOMBACACEAE					
Bombax buonopozense P. Beauv.	6/9	6/10	79/442‡	0/154	10–30
B. buonopozense P. Beauv.	5/5	1/4	3/19	0/79	10
B. buonopozense P. Beauv.	4/5	1/3	2/40	0/51	10
Ceiba pentandra Gaertn.	4/10	5/9	27/319	0/274	10–30
C. pentandra Gaertn.	5/5	3/3	8/96	—	10
Adansonia digitata Linn.	12/17	12/13	39/101	—	20
EUPHORBIACEAE					
Ricinodendron africanum Muell. Arg.	18/25	0/5	—	0/154	20
MORACEAE					
Artocarpus incisa Linn.	4/5	0/5	—	0/31	20
A. incisa Linn.	9/10	0/5	—	0/150	20
Ficus exasperata Vahl.	5/14	0/5	—	0/150	20
RUBIACEAE					
Canthium glabriflorum Hiern.	2/5	0/5	—	0/124	10–30
APOCYNACEAE					
Alstonia congensis Engl.	23/37	0/5	—	0/150	20
Funtumia elastica Stapf.	8/13	0/5	—	0/150	20
ARACEAE					
Xanthosoma sagittifolium Schott.	15/15	0/5	—	0/165	20

* The usual number of infective insects used on the cacao controls was 10 per plant and on the alternative host 30 or 50 per plant. The chance of infection was therefore at least 3 to 1 in favour of the alternative host.

† Diagnosed on symptoms.

‡ One plant gave consistently a high transmission rate totalling 65/91.

may be accompanied by a decline in virus concentration, for it became increasingly difficult to get transmissions to cacao, and no transmission has been achieved from plants infected for more than a year.

Of the hosts in the Sterculiaceae, *Cola cordifolia* R.Br. developed conspicuous

vein banding (Pl. 11, fig. 2) on the first and second leaves formed after inoculation, but subsequent growth was symptomless. As with *Ceiba pentandra*, there was a decline in virus concentration, as judged by vector transmission rates, in the chronic phase. Two experiments were completed with *Erythropsis barteri* K. Schum. The single seedling which became infected showed no symptoms.

The host range of viruses 1 C and 1 M

The virus discovered at Kpeve on the British Togoland-Gold Coast frontier and tentatively termed *Theobroma* virus 1 C (Posnette, 1947) differs in not causing swellings and in several other respects from virus 1 A. Virus 1 M (Todd, unpublished), found at Mampong in the Akwapim hills, differs from 1 A in that infected plants recurrently produce growth flushes with leaf symptoms followed by several symptomless flushes, and also in the pattern and intensity of the mosaic. Because of their distinctive symptoms these viruses were chosen from the large number available for alternative host experiments in the expectation that their host range might be different from that of virus 1 A.

Table 2. *Experiments with cacao viruses 1 C and 1 M; thirty vectors* (Pseudococcus njalensis) *per plant throughout*

Plant species tested	Virus	Infection rate		Back transmission tests to cacao	
		Cacao controls	Alternative host	From infected plants	From healthy plants
Tiliaceae					
Desplatzia lutea A. Chev.	C	13/20	0/5	—	0/28
Sterculiaceae					
Cola chlamydantha	C	4/5	0/8	—	0/48
C. chlamydantha	M	15/20	0/14	—	0/73
C. cordifolia	C	24/43	1/39	2/8	0/293
C. cordifolia	M	32/54	1/48	1/10	0/257
C. nitida	C	3/5	0/5	—	0/43
C. verticillata Stapf.	M	2/10	0/6	—	0/21
Sterculia rhinopetala	C	4/8	8/16	28/137	0/45
S. rhinopetala	M	10/10	6/12	56/170	0/45
S. tragacantha	C	17/36	0/39	—	0/200
S. tragacantha	M	14/14	4/14	5/40	0/53
Bombacaceae					
Adansonia digitata	C	6/10	3/17	5/15	0/94
A. digitata	M	7/20	2/14	2/13	0/76
Bombax buonopozense	C	15/22	1/16	1/4	0/98
B. buonopozense	M	5/10	0/3	—	0/24
Ceiba pentandra	C	12/16	2/34	3/10	0/160
C. pentandra	M	9/12	2/12	2/36	0/69
Malvaceae					
Thespesia lampas	M	8/10	0/18	—	0/29

The species listed in Table 2 were tested by feeding thirty infective mealybugs (*Pseudococcus njalensis*) on each seed or young seedling and on cacao beans as

controls, so that the susceptibility of the host plant relative to that of cacao could be estimated. A comparison of Table 2 with Table 1 shows differences in host range between virus 1 A, 1 C and 1 M. The two *Sterculia* spp. are apparently immune to virus 1 A but susceptible to 1 M, whereas only *S. rhinopetala* is susceptible to virus 1 C.

Cola cordifolia and the species in the Bombacaceae were highly resistant to viruses 1 C and 1 M. Only a few of the plants colonized became infected, and their virus content was low, as judged by the transmissions from them to cacao. Transmissions were obtained from only three *Adansonia digitata* seedlings, although a further nine seedlings developed vein-banding symptoms.

Symptoms

Seedlings of *Sterculia rhinopetala* developed pale green circular spots up to 1 cm. in diameter on each side of the midribs on the first leaves formed after infection with virus 1 C. These spots were caused by the clearing of the microscopic veins. The next leaf showed a large area of clearing on one side of the lamina and sometimes yellow banding of the main veins; as the cleared area did not grow, the leaf became twisted. The leaves formed in subsequent growth were symptomless.

With virus 1 M, seedlings of *S. rhinopetala* developed bright yellow vein banding in the first leaf formed after infection and subsequent leaves were symptomless.

The seedlings of *S. tragacantha* infected with virus 1 M developed no symptoms, and the presence of the virus was detected only by transmission to cacao.

The host range of a Nigerian cacao virus

None of the Nigerian cacao viruses protects plants against the Gold Coast viruses, so despite some similarities in symptoms, they must be regarded as distinct viruses with possibly different host ranges. Because of its high rate of transmission in experiments with a range of vectors, one of the viruses from the outbreak at Offa Igbo was selected for host range experiments from the eight Nigerian cacao viruses being studied at Tafo.

Potential host plants were tested as young seedlings, usually in the four-leaf stage, using thirty vectors per plant, and cacao beans, each with ten vectors, as controls. When symptoms had developed on the controls, the indigenous plants were infested with virus-free mealybugs, and transmission from them to cacao attempted. The results given in Table 3 show that the Offa Igbo virus behaved similarly to virus 1 A, except that *Erythropsis barteri* was not infected.

Ceiba pentandra was the species most susceptible to the Offa Igbo virus, being almost as susceptible as cacao. Symptoms were of the vein-banding type, but brighter yellow than those caused by virus 1 A and recurrent on consecutive growth flushes. No stem necrosis was observed. Mortality among young *C. pentandra* seedlings was high, but cannot be attributed with certainty to the virus (see Field Tests).

Table 3. *Experiments with Offa Igbo (Nigeria) cacao virus*

Plant species tested	Infection rate: Cacao controls (10 vectors)	Infection rate: Alternative host (30 vectors)	Back transmission tests to cacao (20 vectors each): From infected plants	Back transmission tests to cacao (20 vectors each): From healthy plants
Caricaceae				
Carica papaya Linn.	3/10	0/5	—	0/56
Tiliaceae				
Cistanthera papaverifera A. Chev.	4/5	0/5	—	0/100
Sterculiaceae				
Cola chlamydantha	2/13	0/5	—	0/58
C. cordifolia	6/6	1/5	4/42	0/73
C. nitida	5/5	0/5	—	0/86
Erythropsis barteri	4/5	0/5	—	0/11
Pterygota macrocarpa K. Schum.	4/4	0/5	—	0/92
P. macrocarpa K. Schum.	4/12	0/5	—	0/95
Sterculia rhinopetala	6/8	0/5	—	0/132
S. tragacantha	2/4	0/5	—	0/45
Bombacaceae				
Bombax buonopozense	1/10	2/5	3/57	0/61
B. buonopozense	5/10	1/4	11/20	0/68
Ceiba pentandra	1/5	5/5	8/31	—
C. pentandra	4/10	5/5	23/49	—
Malvaceae				
Gossypium hirsutum Linn.	4/5	0/5	—	0/169

Bombax buonopozense was more resistant than *Ceiba pentandra* to the Offa Igbo virus, but from one of the infected seedlings a high rate of transmission was obtained. Symptoms were confined to slight vein-clearing.

Cola cordifolia was very tolerant and symptoms, a vein banding, were restricted to a single leaf.

Field tests

(*a*) *Gold Coast*

Concurrently with the experiments described above, indigenous plants suspected of being infected in the field were tested by transferring vectors from them to cacao test plants. When these wild plants were growing far distant from the laboratory, the cacao test plants were carried as germinating beans in specimen tubes.

The first indigenous plant shown to be a host of cacao virus was discovered in this way. Leaf symptoms (Pl. 11, fig. 3) suggesting a virus infection were noticed on suckers growing from a stump of a *Cola chlamydantha* K. Schum. tree growing in an outbreak of swollen shoot near Wiawso in the Western Province of the Gold Coast. Vectors were fed on detached leaves and stem, and a virus transmission to cacao was obtained. A second stump of *C. chlamydantha* was tested by transferring insects from a natural infestation of *Pseudococcus njalensis* to twenty-five cacao beans, of which ten became infected. The symptoms which developed on the test plants were identical with those on the cacao trees in the outbreaks concerned, and further tests

confirmed that the same virus was present in the *C. chlamydantha* as in the cacao.

C. chlamydantha is a small understorey tree in the forests of parts of the Western Province of the Gold Coast, Ivory Coast and Liberia. It is usually unbranched, with a straight stem reaching a height of 40 ft. and a crown of large palmate leaves, up to 2 ft. in length, borne on long woody petioles up to 5 ft. long. The leaves are glabrous, dark green, leathery and very long-lived.

Two independent surveys were made to determine the degree of infection in *C. chlamydantha* trees growing in the Wiawso district. A natural infestation of mealybugs was usually found on the *C. chlamydantha* plants examined, except on young seedlings less than 2 ft. tall. Trees were felled and mealybugs transferred from their feeding sites (usually inside the bracts of the terminal bud) to cacao beans. In the absence of a natural infestation, virus-free mealybugs were applied to detached stem pieces. Different species of mealybug were kept separate and removed from the beans for identification before the latter were planted in the greenhouse. In addition to the known vectors, *Pseudococcus njalensis*, and *Ps. citri* (Risso), transmissions were obtained with *Paraputo ritchiei* Laing, and with *Pseudococcus masakensis* Green.

In the first survey, of thirty-two *C. chlamydantha* plants tested, sixteen were infected. Infected trees were found not only in cacao farms with swollen shoot outbreaks, but also in secondary 'bush' regenerating after food-crop farming and in a forest reserve about half a mile from the nearest cacao. The second survey therefore concentrated on *C. chlamydantha* trees in forest, to find whether this species is a primary host of the virus or becomes infected from cacao. Of eighty trees tested, transmissions were obtained from thirty-two, of which twenty-five were growing in a forest reserve, and seven were in secondary forest, about 3 miles and 1 mile distant respectively from the nearest infected cacao.

Except on suckers from regenerating stumps, only leaf symptoms of a vague chlorotic blotching were seen on infected trees, some of which had shortened internodes and scaly or gnarled stems, while others appeared normal.

C. chlamydantha does not occur in the Eastern Province of the Gold Coast where the swollen shoot virus 1 A is rife, but *C. pentandra* and *C. cordifolia* are very common as shade trees in cacao farms. Political circumstances have prevented the extensive testing of these trees except on land belonging to the Institute. Both *C. pentandra* and *C. cordifolia* (Pl. 12, fig. 1) grow into very large trees, the felling of which would cause serious damage to cacao underneath. Initial tests were therefore done by feeding vectors on pieces of root excavated from suspected trees associated with swollen shoot outbreaks. No transmission to cacao was obtained from seven *C. pentandra* and nine *C. cordifolia* trees tested in this manner, and so felling was resorted to in an area where most of the cacao was already dying of swollen shoot.

Immediately after felling, each tree was searched for vectors and any found were transferred direct to cacao beans. Succulent young shoots were then taken to the

laboratory and infested with virus-free vectors which were transferred to cacao bean test plants after 24 hr. feeding.

This work is still in progress, but up to the time of writing transmission has been obtained from only two *C. pentandra* trees out of fifty-seven tested. None has been obtained from twenty *C. cordifolia* trees, but a *C. cordifolia* seedling with virus 1A symptoms has been discovered in a swollen shoot outbreak.

Plants belonging to the following species, which often occur in cacao farms where swollen shoot is present, have been found with symptoms suggestive of virus diseases, but attempts to transmit virus from them to cacao, using mealybugs, have all failed: *Solanum verbascifolium* Linn., *Pouzolzia guineensis* Benth., *Vernonia amygdalina* Del., *Celtis Soyauxii* Engl., *Ficus capensis* Thunb., *F. exasperata* Vahl., *Funtumia elastica* Stpf., *Terminalia ivorensis* A. Chev., *T. superba* Engl. & Diels.

(*b*) *Nigeria*

As shown in Table 3, the host range of the cacao virus from the outbreak of swollen shoot at Offa Igbo in Nigeria was investigated at Tafo, and *Ceiba pentandra*, *Bombax buonopozense* and *Cola cordifolia* were proved to be susceptible. In May and June 1948, the Offa Igbo outbreak was surveyed to determine whether infected plants of these or other species were present. The distance (about 5 miles) of this outbreak from the nearest known other outbreak of swollen shoot, and the fact that the Offa Igbo virus is known only from this one outbreak, suggested a local origin in a wild host; but as the outbreak covers about 100 acres and is therefore probably of long duration, the original source might already have been eliminated.

A preliminary survey of the area revealed too large a number of possible host plants for all to be tested with the limited supply of vectors available. There were many trees of the edible cola (*Cola nitida* A. Chev.), some of which were unhealthy and had symptoms suggestive of a virus disease. Two principles were adopted in the selection of plants for virus tests. Primarily, attention was given to species already known to be susceptible to cacao viruses; in addition, all plants showing mosaic, vein-clearing or vein banding were tested.

Vectors were found on only one wild plant (*C. nitida*) so that direct transference of vectors from wild hosts to cacao, the method used successfully to demonstrate infected *C. chlamydantha* trees in the Gold Coast, was limited to this plant. Young shoots and leaves were collected from the suspect trees, taken to the laboratory and infested with mealybugs previously starved for about 24 hr. Specimen tubes sealed with muslin were used as feeding cages, with a layer of damp sand in the bottom of each tube to maintain humidity. The insects were given a feeding period of 18–24 hr. on the suspect material before they were transferred to dissected cacao beans in block watch-glasses. After about 24 hr. the beans were disinfested with nicotine and planted in a greenhouse; 109 beans were colonized, each with twenty insects.

No *Bombax buonopozense* or *Cola cordifolia* plants were found in the outbreak and, as shown in Table 4, virus was transmitted only from the cacao controls.

TABLE 4. *Field tests in Offa Igbo (Nigeria) outbreak*

Host species	No. plants tested	Infection rate in cacao test plants
Cola nitida	8	0/64
C. togoensis	1	0/4
Sterculia tragacantha	1	0/3
Ceiba pentandra	3	0/28
Solanum sp.	2	0/4
Combretum sp.	1	0/2
Theobroma cacao controls	3	1/1, 1/1, 1/2

The failure to transmit virus from these indigenous plants might seem, considering the transmission from the cacao controls, to prove that they were not infected. However, subsequent results with one *Ceiba pentandra* plant illustrate that this conclusion cannot be drawn with certainty. This plant was either a seedling about 3 years old which had been cut back several times, or a self-rooted cutting from an older plant. It was growing near the centre of the outbreak where most of the cacao trees had died, and when discovered it was showing vein-banding symptoms exactly like those on *C. pentandra* seedlings infected in the Tafo experiments. It was transplanted and kept in a greenhouse at Ibadan. Most of its younger leaves fell, but sixteen tests were done, each with twenty vectors, from the stem and old leaves without obtaining a transmission to cacao. The stump of the seedling was brought to Tafo and regenerated in a greenhouse. From the stem and first leaves, which showed no symptoms, no virus was transmitted to cacao in twenty-four attempts with mealybugs. Then a flush of leaves with symptoms was produced, and of twenty-two attempts to transmit virus to cacao, eleven were successful. The symptoms in the cacao test plants were the same as those in the cacao at Offa Igbo.

This result emphasizes the difficulty of interpreting negative results in transmissions from alternative hosts in the field. The known wild hosts of cacao viruses are tolerant. Judging from the rates of transmission in our experiments, the virus concentration usually falls to a low level after the plants have been infected for a few months. The new shoots arising from a coppiced stump often show a recurrence of symptoms, presumably associated with a higher virus content. This probably explains the negative results with *C. pentandra* in Nigeria and the positive transmissions from the same plant at Tafo after it had been cut back.

This experience with *C. pentandra* suggests that *Cola nitida* trees may also be infected at Offa Igbo, but this seems unlikely because we have failed to infect seedlings of *C. nitida* at Tafo.

The practical conclusion to be drawn from these experiments is that, since one of the known wild hosts of Nigerian cacao virus can become infected in the field, the others may also, and should therefore be eradicated from cacao farms wherever practicable, and certainly from farms where diseased cacao has been felled. *C. pentandra*, *Bombax buonopozense*, *Adansonia digitata* and *Cola cordifolia* are not common in mature cacao farms around Ibadan, but as coppiced stumps, on which

the regenerating shoots are likely to be repeatedly cut back by the farmer, they could constitute a reservoir of virus.

Although *C. togoensis* has been shown not to be a host of these cacao viruses, it is of interest to record that a virus disease of *C. togoensis* was discovered near Ibadan (Nigeria). Leaf symptoms of the vein-banding type were noticed on a regenerating stump, and transmission by grafting to *C. togoensis* seedlings was obtained at Tafo. Repeated attempts have failed to transmit this virus to cacao with *Pseudococcus citri* and *Ps. njalensis*, and no symptoms have developed on seedlings of *Cola cordifolia*, *C. chlamydantha* and *C. nitida* to which transmissions have been attempted by grafting.

Discussion

The mealybug vectors of cacao viruses have a wide range of food plants—*Pseudococcus njalensis* alone has been taken from over a hundred different species of plants on the Gold Coast and *Ferrisia virgata* Ckll. has fed and bred freely on all the plants on which it has been placed; this ease of establishment of the insect vector has compensated in the host-range studies for the lack of graft-compatibility between cacao and indigenous plants, and has obviated the use of dodder.

The investigations described in this paper show that, though the vectors feed on so many species, the host range of the viruses used is apparently restricted to plants in the Sterculiaceae and Bombacaceae.

In their reaction to cacao viruses, the indigenous host plants differ conspicuously from cacao in two main features, symptom expression and ease of virus transmission. Leaf symptoms with some species show a general resemblance to those on cacao but the tendency is for symptoms to occur only when plants are newly infected. Transmission by vectors from these species to cacao is usually difficult and often impossible in the chronic (symptomless) phase. These features suggest a virus concentration much lower than in cacao, and this would account for the difficulty experienced in obtaining transmission in the field from trees that may have been infected for long periods. The role of dandelion in outbreaks of yellow mosaic in lettuce (Kassanis, 1947) seems to be analogous.

The importance of one indigenous host, *Cola chlamydantha*, seems clear; the presence of other infected wild hosts outside the geographical range of *C. chlamydantha* could be inferred from the following facts:

(*a*) The cacao viruses of the *C. chlamydantha* region are distinct from those of other areas in the Gold Coast and Nigeria, and there is no evidence that the latter have been derived from the former.

(*b*) Infected cacao has been found in forest reserves where spread from other infected cacao is highly improbable (e.g. near Mpraeso, Kwahu, and near Bekwai, Ashanti); in an area where there is little swollen shoot, outbreaks are most prevalent in cacao growing near the boundaries of a forest reserve (Atewa Range).

(*c*) At Tafo, where tree-to-tree spread in cacao has been effectively controlled by

roguing, so that peripheral spread has ceased in 317 out of 341 outbreaks treated between 1940 and 1948, new outbreaks have occurred at a slightly increasing rate each year. It was suggested (Posnette, 1943) that these new outbreaks were caused by spread either from wild hosts growing among the cacao or by wind-borne insects from infected cacao outside the Institute's land. The latter alternative now appears the less likely, because, although wind dispersal of mealybugs has been proved (Strickland, 1949), the removal of the bulk of the infected cacao from the adjoining land in 1945 and 1946 has not reduced the incidence of new outbreaks; and also because there is no gradient of infection with a higher density of outbreaks on the boundaries and a lower density farther away from the supposed source of infection.

Of the plants known to be susceptible to virus 1 A, *Bombax buonopozense* appears to be unimportant as a field host because it is rarely a host of the known vectors; in fact *Pseudococcus njalensis* has never been collected from this species and did not breed on it when it was artificially infested. *Adansonia digitata* is seldom found in association with cacao, and usually grows in areas too dry for the crop. *Ceiba pentandra* and *Cola cordifolia*, on the other hand, are commonly found in cacao farms and are favoured host species of the vectors.

There is a fairly even distribution of *Ceiba pentandra* and *Cola cordifolia* trees on the Institute's land at the rate of about one per acre and one per 2 acres respectively; some of them are near swollen shoot outbreaks and others are not, as would occur if only some were infected or, as they are common trees, if none was. Both species are susceptible to virus 1 A and are hosts of the vectors, so it is improbable that all can have escaped infection in a region where the disease has been widespread in cacao. Indeed, field tests proved that at least some are carrying the virus, and the low transmission rate obtained from these suggests that many others from which no transmission occurred may also be infected. Whatever the number already infected, all are potentially dangerous to cacao, for they are liable to become infected, although they would remain symptomless and the fact might be obvious only from disease occurring in neighbouring cacao.

Unfortunately, wild hosts of cacao viruses are not as amenable to eradication as the chokecherry host of 'X' virus disease of peach (Berkeley, 1941) or the barberry host of cereal rusts. *Ceiba pentandra* is one of the largest trees of the forest, attaining a height of over 200 ft., while buttresses at the base of the trunk make felling a difficult and expensive operation. No use has yet been found locally for the timber; it cannot even be used for firewood. After felling, the stumps of both *C. pentandra* and *C. cordifolia* sucker freely. Immediate eradication depends on the discovery of suitable poisons, and experiments to this end are in progress, but if growers can be convinced that these trees are a danger to their cacao much trouble could be avoided in future by the eradication of seedlings and saplings before they reach an unmanageable size.

Differences in host-virus relationship among the various alternative hosts are already evident. It is generally true that virus transmission by vectors is increasingly

difficult as the infection becomes chronic. *C. chlamydantha* is, however, a notable exception. Transmissions are obtainable from groups of *C. chlamydantha* plants in the field, as readily from mature trees as from seedlings and coppiced plants. 'Outbreaks' occur in *C. chlamydantha* populations as with cacao, and there is little doubt about the importance of this species as a reservoir of virus. The other species found to be virus hosts are apparently dangerous in a less spectacular and more pernicious manner.

Alternative host studies are proving of value, together with vector studies and protection tests, in cacao virus taxonomy. This will be discussed fully elsewhere and only two examples are given here. *C. chlamydantha* is easily infected with any Western Province or Ivory Coast virus yet used and with virus 1 J from Ashanti, but has not been infected with the Eastern Province viruses 1 A, 1 C or 1 M. The suggestion made previously (Crowdy & Posnette, 1947) that virus 1 C is a distinct virus and not a strain related to virus 1 A is supported by the difference in the reaction of *Sterculia rhinopetala*, which is susceptible to virus 1 C but apparently immune to virus 1 A. The host range of virus 1 C and that of 1 M differ in respect of *S. tragacantha*, but both can infect *S. rhinopetala*, perhaps indicating a closer relationship to each other than to virus 1 A. It is interesting to note corroborative evidence of similarity between 1 C and 1 M, two viruses with very different symptoms, in their vector range.

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Fig. 1. Spine suppression by virus 1 A on *Ceiba pentandra* stem; normal stem on right.

Fig. 2. Vein-banding symptom of virus 1 A in *Cola cordifolia* leaf (seedling).

Fig. 3. Vein-banding symptom of virus 1 F in leaflet of *Cola chlamydantha*.

Fig. 1. A mature tree of *Cola cordifolia* some 150 ft. tall. Tallest trees in right background are *Ceiba pentandra*.

THE IDENTITY OF THE SWEDE MIDGE, WITH NOTES ON ITS BIOLOGY

By H. F. BARNES, M.A., Ph.D.

Entomology Department, Rothamsted Experimental Station

A brief historical summary is given of the literature concerning *Contarinia nasturtii* Kieffer, *C. torquens* de Meijere and *C. geisenheyneri* Rübsaamen, three gall midges (Cecidomyidae) which have been associated respectively with leaf damage on *Brassica* spp. in England, identical malformation on the Continent and swollen and closed flowers of *Brassica* spp. in Europe.

Following the discovery in England of the true *Contarinia nasturtii* causing swollen and closed flowers of *Rorippa amphibia*, preliminary experiments have shown that midges from this source will also cause similar damage to swede flowers and that they are the same species as those attacking *R. amphibia* blossom in the Netherlands.

Further preliminary experiments have shown that swede midge derived from rape leaf axils will also cause identical damage to radish flowers in addition to breeding successfully on the leaves of turnip, swede, cabbage and radish.

Male midges derived from *Rorippa* blossom have been mated with females from rape leaf axils, and their offspring have been reared on *Rorippa* flowers and on turnip leaves.

This biological evidence confirms the previous supposition based on morphological grounds that the swede midge is *Contarinia nasturtii* Kieffer and will cause either flower or leaf damage. Consequently, *C. torquens* de Meijere and *C. geisenheyneri* Rübsaamen must remain synonyms.

The shortest time for a generation (from parent to first offspring midge) to develop was 24–39 days in an unheated open glasshouse during June–July in 1949 at Harpenden. Under the same conditions, the shortest time for the next generation from late July to August was 29–32 days. *C. nasturtii* is the first *Contarinia* species in which unisexual families have been discovered.

Introduction

When Taylor (1912) discovered the swede midge causing 'cabbage top' (i.e. the 'many-necked' and 'crumpled leaf' condition) on swedes, Kieffer identified it as *Contarinia nasturtii* Kieffer, which he had originally described (1888) 24 years previously from swollen and closed flowers of *Nasturtium palustre* DC., now known as *Rorippa islandica* (Oeder) Borbas.

Before Taylor's discovery of the swede midge, de Meijere (1906) had described as *Contarinia torquens* the midge that was responsible for blindness and distortion of the hearts of savoy cabbage in Holland—the 'draaihartigheid', 'krauselkrankheit' and 'drehherzigkrankheit' damage in Holland and Germany.

The 'cabbage top' of swedes in England was quite obviously the same type of damage as the 'draaihartigheid', etc. of cabbages on the Continent, but in spite of Theobald's (1912) suggestion that *C. nasturtii* and *C. torquens* were one and the same

insect it became customary for continental workers to use the name *C. torquens* while English workers retained the name *C. nasturtii*.

P. Bovien, in correspondence in 1929, suggested that *C. nasturtii* was responsible for a lot of the damage ascribed to *C. torquens* in north Germany. About this date the writer expressed the opinion, in correspondence to several workers on these midges, first that *C. torquens* was a biological race of *C. nasturtii* and then later that the two species were synonymous. This synonymy, based on the examination of slides of *C. torquens* and *C. nasturtii*, was accepted by several investigators (see Balachowsky & Mesnil, 1936).

But the German authority Rübsaamen (Rübsaamen & Hedicke, 1925–39) retained the two species, stating that *C. nasturtii* caused swollen flower damage on *Nasturtium palustre* (*Rorippa islandica*), *N. silvestre* (*Rorippa sylvestris* (L.) Besser), *N. officinale*, *N. amphibium* (*Rorippa amphibia* (L.) Besser), *Raphanus sativus* and *R. raphanistrum*, whereas *Contarinia torquens* lived in the leaf axils of *Brassica* spp. Incidentally, Rübsaamen sunk his own species *Contarinia perniciosa* as a synonym of *C. torquens*.

In addition, Rübsaamen (1917) had previously described *C. geisenheyneri* from the swollen and closed flowers of various Brassicae, but the present writer after examining slides of this species in 1928 came to the conclusion that *C. geisenheyneri* was also synonymous with *C. nasturtii*. P. Bovien in Denmark, about this date, obtained biological evidence of this synonymy by placing several hundreds of *C. nasturtii* reared from 'crumple leaf' on swedes into a cage containing swedes in flower and obtaining typical *C. geisenheyneri* flower damage. It should be stated here that Kieffer (1893) had many years previously recorded *C. nasturtii* from the blossom of rape.

Noll, Roesler & Benner (1942) reared midges from the blossom of wild radish (*Raphanus raphanistrum*), *Brassica rapa* L. and *B. oleracea* L. for comparison with those reared from typical 'drehherzigkrankheit' damage. Examination of this material satisfied Dr W. Hennig that only one species, *Contarinia nasturtii*, was involved, and that *C. torquens* was not a valid species although there were small differences in the antennae and genitalia of the males among the different rearings. Thus the view that *C. torquens* and *C. geisenheyneri* were synonymous with *C. nasturtii* received additional support.

A few years ago it was suggested (Barnes, 1946) that an attempt should be made to transfer *C. nasturtii* reared from the blossom of *Rorippa islandica* (*Nasturtium palustre*) to cultivated *Brassica*, thus making quite sure that Kieffer was correct in his original identification of Taylor's swede midge as *Contarinia nasturtii* and incidentally proving that one species of gall midge can cause both blossom and leaf damage.

In 1949, Dr P. Bovien carried out the reverse of his 1928 experiment and wrote on 26 September as follows: 'In order to see if gall midges bred from galled cabbage flowers would be able to produce deformation of the heart in young cabbage plants,

the following experiment was set up in July 1949. Galled flowers from cauliflower plants grown for seed production were collected on 7 July and placed on soil in an emergence cage. From 18 to 27 July about 300 midges emerged. Thirty young cauliflower plants were planted in each of two cages in the insectary and into one of these the midges were released as they emerged. The other cage was kept closed all the time. One of the plants which had been exposed to attack showed symptoms on 25 July and numerous newly hatched larvae were present in the heart. On 2 August ten plants were examined. Seven of them showed distinct symptoms (distortion of the heart leaves) and in all 115 larvae were found in them (from five to twenty-seven/plant). The final result was that eighteen of the thirty plants which had been exposed exhibited the typical symptoms. The control plants were all entirely free from attack.'

The object of the present paper is to give the results of experimenting on the host plant range of midges obtained from two sources, namely, the blossom of great watercress (*Rorippa amphibia* (L.) Besser) and the leaf axils of rape, in an attempt to establish the identity of the swede midge on biological evidence.

Experiments with *Contarinia nasturtii* obtained from the blossom of great watercress (*Rorippa amphibia*)

In 1948, on 3 July, some swollen and closed flowers of *Rorippa amphibia* were received from Dr W. Docters van Leeuwen in Holland. They contained sulphur-yellow full-grown larvae which were put to breed in the insectary. On 6 July identical galled flowers of *R. amphibia* were discovered near the river at Bedford. These, and others collected in the same locality on 11 July, were also put to breed. From the Dutch material twenty-three female midges were reared between 24 July and 16 August, of these eighteen emerged between 25 and 27 July. From the Bedford material forty males and forty-seven females emerged between 28 July and 21 August, the peak emergence being 29–31 July when thirty-two males and thirty-five females emerged.

One female of the Dutch material was given the opportunity of mating in a small glass tube with a Bedford male on 30 July. This it did quite readily, thus confirming that the Dutch and English midges were the same species. It is without doubt the true *Contarinia nasturtii* Kieffer, and this is probably the first occasion on which it has been found and reared from *Rorippa* in England.

On 23 August identical galled flowers were discovered on *R. amphibia* which was being grown in Rothamsted Lodge garden. This attack may have come from nearby infested turnips.

In 1949, no further midges emerged from the Dutch material, but an additional fifty-three males and sixteen females were obtained from the Bedford material between 23 May and 5 July, chiefly between 27 May and 14 June; while four males and twenty females emerged between 2 June and 2 July from the *Rorippa* flowers collected from Rothamsted Lodge garden on the previous 23 August.

Successful matings in tubes were made on eight occasions between females derived from the Rothamsted material and males from the Bedford stock.

Some of these midges were used in attempts to discover whether they would breed successfully on the leaves of swede, turnip and cauliflower, and on the flowers of swede as well as on *Rorippa amphibia* blossom. Owing to the comparative shortage of this material, together with the possibility of the occurrence of unisexual families, usually only one female was used in each experiment. In spite of many of the plants being heavily infested with aphids and sometimes *Plutella maculipennis* L., midges were successfully reared on swede flowers, which remained closed and swollen, and also on *Rorippa* flowers. Egg laying was observed on unopened swede blossom on 13 June while the first G_1 midges in one cage emerged on 12 July and in the other on 13 July. The generation took twenty-nine and thirty days respectively in the two experiments. Emergence continued for 2 and 4 days. In one cage two males and fifteen females emerged and in the other forty-eight males and one female. In one cage of *Rorippa* blossom set up on 21 June G_1 midges started emerging on 14 July twenty-three days after the introduction of the midges and continued for 9 days. The occurrence of a family consisting of forty-eight males and one female indicates the occurrence of unisexual families.

Experiments with swede midge obtained from the leaf axils of rape

A stock of rape side-shoots infested with swede midge larvae collected from Whalton, Northumberland, on 13 September 1948 was obtained from Dr M. Cohen. Between 4 June and 14 July 1949, 120 males and 333 females emerged from this material. There was a definite peak of emergence on 3 and 4 July. Up to these dates only ten males and eighteen females had emerged, on 3 and 4 July sixty-eight males and 182 females emerged, and subsequently a further forty-two males and 133 females. The preponderance of females again suggests some departure from a 50:50 sex ratio. Evidence of delayed emergence was obtained by the fact that a few additional emergences from this material took place on 26 and 28 August, no living food plant ever having been in these emergence pots.

Some of these midges were used in attempts to discover whether the swede midge would breed successfully on turnip, radish, swede and cabbage leaves, and also on radish and *Rorippa amphibia* flowers. These experiments were started between 29 June and 5 July, several females (2–6) being liberated with males in each cage. The midges had no choice of plant on which to oviposit, the pots each containing only one kind of plant. Oviposition was observed on the *Rorippa* blossom. Typical swollen and closed flower galls containing nearly full-grown larvae were observed in one pot containing radish in flower on 20 July. Midges were reared successfully in three cages containing young turnip plants, in three cages containing young cabbage, in one cage with young swede plants, in one cage with young radish plants, in the leaf stage only, and in one cage from radish flower galls. Emergence of G_1 midges started from turnip leaves 24, 26 and 28 days respectively after the

introduction of the parent midges, from cabbage leaves on the 25th, 26th and 29th days, from the swede leaves on the 24th day, from the radish leaves on the 24th day and from the radish flowers on the 28th day after the beginning of the experiment.

The sex ratio of these rearings, in spite of not being from solitary females, gave further indication of the occurrence of unisexual families. Thus from one turnip cage 110 males and fifty-seven females were reared, from another two males and twenty-five females, while from one cage of cabbage no males and thirty-nine females were bred.

A further set of experiments were set up on 29–31 July, in each case using young plants of cabbage, swede, turnip or radish which had been sown on 4 July, and grown in the open unheated glasshouse under muslin cages from the time of sowing in order to avoid aphid attack. Only one female was introduced into each cage with one or two males. The midges, in every case derived from grandparents reared from rape side-shoots, were of mixed immediate host-plant origin (cabbage, radish, swede or turnip), although without exception reared from leaf damage. Their consorts did not always come from the same host plant.

Emergence of the G_2 midges started on 28 August: on radish 30 and 31 days after insertion of the parent female midge, on swede 29, 30 and 32 days after the introduction, and on turnip 30 days after the beginning of the experiment. Although the number of emergences was not large and very suggestive of only a partial emergence, only midges of one sex were obtained from each experimental pot. Thus from one radish pot forty-nine females were obtained but no males. This is additional evidence that *Contarinia nasturtii* breeds by means of unisexual families.

In addition to these G_2 experiments, a second generation was obtained from a few of the G_1 experimental pots. Thus from a swede pot thirteen males and eighty-four females were obtained between 24 August and 12 September.

Third generation experiments were set up on 30 and 31 August using solitary females derived from one family. Galls on turnip and swede leaves were clearly visible by 8 September. Partial emergences took place in three experimental pots between 28 September and 12 October, starting 28, 34 and 43 days respectively after the insertion of the parent midge.

Experiments in crossing the *Rorippa* blossom midge with the rape leaf axil midge

In spite of the inherent difficulties of obtaining virgin females at the same time as suitable males for cross-mating experiments, on three occasions mating was observed between females reared from rape side-shoots and males from the Bedford *Rorippa amphibia* blossom, twice in glass tubes and once in a muslin cage. Mating of *Contarinia* spp. does not take place so readily in glass tubes as in the case of *Dasyneura* spp. This may be associated with the simple claws of the *Contarinia*, in contrast

to the toothed claws of *Dasyneura* spp., preventing a really good foothold on the smooth surface.

These three rape females which had been seen mating with *Rorippa* males, as well as two other rape females, were put (the latter with *Rorippa* males) into separate cages of either *R. amphibia* plants in blossom or young turnip plants which only had leaves. A small number of G_1 midges was obtained in two cages, one containing *Rorippa* blossom, the other turnip leaves, in both these cases the female had previously been seen mating. G_1 from the *Rorippa* blossom consisted of five females only, that from the turnip leaves was seven females only. Galls had been seen on the *Rorippa* blossom 7 days after the female had been put into the cage and here emergence took place on the 39th day, while in the turnip cage emergence started on the 31st day.

Discussion

Closely allied species of gall midges are notoriously difficult to distinguish morphologically owing to the overlapping variation in diagnostic characters best seen when a long series is available. In spite of this, that great European gall midge authority Kieffer identified the swede midge that Taylor sent him from England from the leaves of swedes as *Contarinia nasturtii*, which was rather surprising because *C. nasturtii* was originally found causing flower damage on *Rorippa islandica*. Other authorities considered the midge causing leaf damage on *Brassica* spp. as a distinct species (*Contarinia torquens*) and also recognized as a third species (*C. geisenheyneri*) as causing flower damage on *Brassica* spp., although Kieffer had recorded *Contarinia nasturtii* from the flowers of rape. Nevertheless, opinion was veering in some quarters towards the view that the leaf and blossom damage on *Brassica* spp. was caused by one species of gall midge. This was based chiefly on morphological examination and on Dr Bovien's first experiment. Only a few investigators, however, associated this cultivated-brassica midge with the wild blossom-inhabiting *Rorippa* (*Nasturtium*) species; again on morphological comparisons and largely because of Kieffer's original identification of Taylor's swede midge as *Contarinia nasturtii* of *Rorippa* blossom.

There is no evidence that anyone had attempted to prove experimentally that the *Rorippa* spp. blossom midge could breed successfully on *Brassica* spp. and cause both leaf and blossom damage, or that the leaf midge of *Brassica* spp. could live on *Rorippa* spp. blossom or that the flower midge of *Brassica* spp. could live on blossom of *Rorippa* spp. Neither had anyone attempted to cross-mate the midge (or midges) from *Brassica* spp. with the midge from *Rorippa* spp.

The experiments described in this paper have shown that: (1) what must be considered the true *Contarinia nasturtii* Kieffer, derived from *Rorippa amphibia* blossom, will breed on swede blossom; (2) the swede midge derived from rape leaf damage will breed on turnip, radish, swede and cabbage leaves and also on radish flowers; (3) male midges derived from *R. amphibia* blossom will mate successfully

with females derived from rape leaves; and (4) the offspring of such a cross will develop on both the flowers of *R. amphibia* and the leaves of turnip.

Only positive results have been mentioned, since negative ones are not proof in such experiments, owing to the difficulties encountered in getting the plants at the right stage of development for oviposition and successful larval establishment and growth at the moment when the midges are available.

Nevertheless, these experiments provide additional evidence that only one species, *Contarinia nasturtii* Kieffer, is involved, and that it will cause both blossom damage on *Rorippa* spp., *Brassica* spp. and *Raphanus* spp. and also leaf damage on *Brassica* spp. and *Raphanus* spp. *Contarinia torquens* de Meijere and *C. geisenheyneri* Rübsaamen must remain as synonyms. Thus Kieffer's original identification of Taylor's swede midge is vindicated.

Examples of all the above rearings are in the Barnes collection, which is particularly rich in material of *C. nasturtii*. It includes, in addition, a male and females reared from swede flowers in Jutland, Denmark, during 1926 (M. Thomsen); males and females bred from flowers of the wild *Raphanus raphanistrum* L. subsp. *segetum* Clav. (=*R. lampsana* Gaertn.) at Catryp, Holland, collected on 27 August 1946 and emerged at the end of April 1947 in the laboratory (G. van Rossem); males and females from cabbage flowers at Naaldwijk, Westland, Zeeland, Holland, during August 1947 (S. Leefmans); males and females bred from winter rape flowers at Svalof, Sweden, during July 1948 (E. Sylvén); a male and female reared from cabbage leaves at Antony (Seine), France, during August 1926 (P. Marchal); males and females from cabbage leaves at Oud Beierland, Zeeland, Holland, during June 1937 (S. Leefmans); males and females reared from swede leaves in Yorkshire during September 1911 (T. H. Taylor); males and females bred during August 1944 from swede leaves at Dawlish, Devon (D. C. Thomas); and a male and females reared during July 1925 from turnip leaves at Wye, Kent (H. F. B.).

Among the biological facts given in this paper there is strong evidence that *Contarinia nasturtii* breeds by means of unisexual families. Previously this phenomenon was only known to occur among gall midges in three genera, namely *Rhabdophaga*, *Thomasiniana* and *Mayetiola* (*destructor* Say). Its occurrence in the genus *Contarinia* opens up new possibilities in the understanding of the species problem in this genus.

The full host plant range of *C. nasturtii* among Cruciferae still remains to be studied, as well as many other details concerning its biology.

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OBSERVATIONS ON THE BIOLOGY OF *CORYMBITES CUPREUS* F. (COLEOPTERA, ELATERIDAE)

By E. E. EDWARDS, D.Sc.
University College of the Gold Coast, West Africa

and J. R. EVANS, M.Sc.
*University College of South Wales and Monmouthshire, Cardiff**

The life history of *Corymbites cupreus* F. is described, and its economic importance is assessed from field observations and laboratory experiments. The females are on the wing in May and June, egg-laying takes place in June and the incubation period is about 34 days. The larval stage probably occupies 5 years under field conditions and pupation occurs towards the end of July or beginning of August. The imagines emerge in about 21 days, but they do not always remain in their earthen pupal cells until the following spring as do several other Elaterid species. The larvae feed principally on the roots and underground stems of plants, but they can exist on decayed organic matter in the soil. Individually they are far more injurious to potatoes, wheat, oats, barley and other cultivated crops than those of either *Agriotes* spp. or *Athous niger*, but because they are restricted to comparatively high altitudes, they are seldom pests of major importance. Birds and carabid larvae readily feed on them and they are also subject to attack by a hymenopteran parasite and the fungi, *Metarrhizium anisopliae* (Metsch) Sor. and *Synglio-cladium cleoni* (Wize) Petch. All attempts to obtain control of the larvae by the use of these fungi failed.

Introduction

The survey made during 1939–46 of the distribution and economic significance of wireworms in South Wales and Monmouthshire showed that *Corymbites cupreus* F. is a major pest of agricultural crops in these areas. Various species of *Corymbites* are already recognized as important pests, notably in America, Russia, Germany and Japan, but *C. cupreus* F. has been recorded by only three workers as a pest of cultivated crops. All three deal with *C. cupreus*, subspecies *aeruginosus* F. Linnaniemi (1935) states that it was the most important Elaterid pest from 1917 to 1923 in Finland. Saalas (1923) recorded it on barley, oats, rye, potatoes and rape at Kainuu, and Hukkinen (1925) on barley in northern Finland.

Life history

Earlier writers, including Beling (1883), state that beetles which emerge from the pupal cases in late summer and in autumn remain in their earthen pupal cells until the following spring. Horst (1922) quotes Sorauer as saying that the beetles if disturbed and exposed to the atmosphere soon die. In the vicinity of Cardiff in 1939–45, however, beetles transferred from the soil into open-air breeding cages in

* Now at Welsh College of Pharmacy, Cathays Park, Cardiff.

the winter, later copulated and laid eggs. It is possible that beetles normally remain in their earthen pupal cell over the dormant months, but only on one occasion was a pupal case recovered from the earthen cell in which the beetle had remained quiescent over the winter. Horst (1922) records that young Elaterid adults, including *Agriotes obscurus* L. and *Corymbites aeneus* L., were on the wing on warm days in the Kopernitz fields near Rhinesberg, a place in latitude comparable with Cardiff. He recovered other newly emerged specimens during the winter months under heaps of stones in fields, in heaps of dead grass and under fallen leaves. Confirmation was obtained by him from Dr V. Lengerken that *Agriotes obscurus* L. may be found in similar situations during the dormant period. It appears from this literature and from observations made in South Wales and Monmouthshire that the beetle may remain in the winter in the earthen pupal cell or hiding in some sheltered situation, either below or above the ground.

During the winters of 1941–3 adults were collected from various localities at high altitudes in the counties of Glamorgan and Monmouth. The beetles were put in earthenware flower pots, 9 in. in diameter, the drainage holes of which had been covered with a fine gauze to prevent the downward escape of the adults and larvae. The soil was covered with a layer of turf, 2 in. deep, to provide suitable conditions for breeding. The pots were plunged to within $1\frac{1}{2}$ in. of the rim in a bed of ashes and soil in an open-air enclosure. A glass cylinder was fitted in each pot and its open end was covered with perforated zinc. On being placed in these cages, the beetles immediately burrowed into the turf, and did not appear again above the ground in 1941–2 until 3 April, and in 1942–3 until 5 April, when they were observed in both years feeding on the leaves of the grasses growing in the pots. They gnawed large notches out of the leaf margin, leaving a jagged edge.

The amount of damage inflicted by Elaterid adults has been largely overlooked in the past. At Abertridwr and Gelligaer, Glamorgan, *Corymbites cupreus* F. was noted in considerable numbers resting on bracken, while others were seen actually feeding on oat seedlings. Xambeau (1912) records that adults of *C. cupreus* F. and its varieties were numerous on the grass slopes of the high plateaux of Carnigou and la Rouquette at the end of June. Horst (1922) also records considerable feeding by adult Elateridae.

The adults were on the wing in South Wales at the end of May and in early June in 1942, the weather at the time being extremely hot and sunny. They fly like cockchafers (*Melolontha vulgaris* F.), often resting on the fronds of bracken and are exceedingly active on warm days in strong sunlight, but immediately seeking shelter in cool or damp weather. Mating takes place freely on bracken fronds and on grasses, the copulation period lasting from 1 to 2 hr. The female then seeks a sheltered position, usually in turf, where she deposits her eggs. From four adults, two females and two males, placed in one of the breeding cages in the winter of 1942, 156 eggs were recovered on 26 May. Nearly all the eggs were collected at a depth of $\frac{1}{8}$–$\frac{5}{8}$ in. below the surface, but one adult was found dead at a depth of 2 in. with five eggs

close to it. Another three females, transferred into breeding pots on 3 June 1942, laid 246 eggs by 20 June, an average of 82 eggs for each female. The females die soon after laying their eggs, usually with their ovipositors fully extended, while the males may die shortly after copulation or survive until the autumn. Various authors have emphasized that the adults of *Agriotes obscurus* L. will not lay their eggs except in turf covered with grass. *Corymbites cupreus* F. lay their eggs freely on bare ground, if the soil is sufficiently moist.

Females kept in breeding cages with dry soil remained alive until the autumn when they died without laying eggs. The ovaries dissected immediately after death were found to be full of eggs. Eggs may be laid singly, or in groups of four or five, sometimes more, in which cases they are lightly held together by means of a mucilaginous substance covering the egg-shell.

Eggs recovered on 26 May 1942 showed no signs of a developing embryo. These were transferred into small Petri dishes, lined with filter-paper and containing fine earth, and moistened periodically; 96% of the eggs hatched:

Date of hatching	Number of eggs hatched
27 June	27
29 June	22
30 June	1
1 July	41
2 July	40
3 July	20
Total	151

The incubation period for this set of eggs varied between 33 and 38 days.

The eggs collected on 20 June were similarly treated. The hatching results were:

Date of hatching	Number of eggs hatched
20 July	58
21 July	43
22 July	40
23 July	52
24 July	20
25 July	10
27 July	12
Total	235

This gives a hatch of 95·5% and an incubation period from 30 to 37 days.

The egg is more resistant to drought than the first instar larva. A number of eggs and larvae were kept together in Petri dishes containing some soil which was slowly air-dried. All the larvae soon died but, on moistening the soil, all the eggs hatched in due course.

The first larval moult occurs some 6 weeks after the eggs are hatched. The larvae pass through the first winter in the second instar, moulting at the end of April. The results of breeding experiments showed that the larval life of *C. cupreus* F. occupies 5 years.

Fully grown larvae in their first year do not exceed 5 mm. in length, while those in their second, third and fourth year average about 8·25, 14·5 and 24·0 mm., respectively. Little or no increase in length takes place after the fourth year.

The larva moults twice a year, in early spring and in summer. The increase in length after an ecdysis is roughly 2 mm. and moulting involves the splitting of the chitin in the dorsal region of the thorax. The larva gradually works its way out of the exuviae with the aid of its legs, but it often finds difficulty in extricating itself from the old tracheal tubes. At first, the larva is very soft, cream in colour, with reddish brown setae. The first parts to become heavily chitinized, as judged by colour, are the mandibles, pseudocerci and the tarsal claws. Gradually, the rest of the body assumes its characteristic colour, the whole process taking about 3 days from the time of emergence of the larva.

For a detailed study of the pupal stage, fully grown sluggish larvae were transferred from the breeding-pots to cages kept out of doors. Each cage was half filled with soil which at no time was allowed to become excessively moist. Pupation normally takes place in the latter part of July, and the adults emerge about 21 days later. Prior to pupation, the larva forms an earthen-cell with the walls evenly lined with soil particles cemented together by a mucilaginous secretion. Horst (1922) suggests that the cell is formed by the larva revolving about its long axis.

The tips of the mandibles and base of the antennae show signs of chitinization about the 12th day, followed later by the prothorax and legs. About the 20th day, the pupal case splits in the metathoracic region, and with the aid of its legs the imago works its way out of the exuviae. At first the adult is exceedingly delicate and pale in colour but on the 4th day its chitin becomes hardened.

Natural enemies

Ford (1917) points out that as wireworms live underground one would expect them to be fairly free from natural enemies. But Elateridae, both larvae and adults, appear to form a portion of the diet of many birds. Examples given by Collinge (1912, 1913) include the starling (*Sturnus vulgaris*), the blackbird (*Turdus merula*) and the thrush (*T. musicus*), all of which feed Elaterid larvae to their young.

Crows (*Corvus coroné*) were observed at Gelligaer, Glamorgan, alighting on newly ploughed fields and feeding on wireworms turned up with the furrow slices. These fields contained a high proportion of *Corymbites cupreus* larvae, the remainder belonging to the genus *Agriotes*. Several *Corymbites cupreus* larvae were placed together with cereal grain in a shallow box of soil on cultivated ground at Aberdare, Glamorgan, and it was found that thrushes, crows and blackbirds fed readily on them.

Various workers, including Ford (1917), record that Carabid larvae feed on wireworms. In laboratory tests the larvae of three different species of Carabids were fed on wireworms and on breeding them to adult, they proved to be *Pterostichus madidus* Fab., *Nebria brevicollis* Fab., and *Harpalus ruficornis* Fab., respectively.

Earlier writers, including Ford (1917) and Roberts (1919), have noted that wireworms are attacked by *Proctotrupid* larvae. One larva of *Corymbites cupreus* kept in soil under laboratory conditions was parasitized by a hymenopteran, the whole body of the wireworm being full of larvae of the parasite. Unfortunately these parasitic larvae could not be bred out.

Roberts (1919) records wireworms infected by a fungus of the genus *Isaria*. In the present studies, five larvae of *Corymbites cupreus* became infected under laboratory conditions with two distinct species of fungi, four of them with the fungus *Metarrhizium anisopliae* (Metsch) Sor., popularly called Green Muscardine. Extensive work has been done by different investigators on the control of insect pests, including *Cleonis punctiventris*, *Tomaspie varia* and *Rhabdocnemis obscura*, by means of this fungus (Morrill & Back, 1912; Petch, 1925) but with little success. The other fungus proved to be *Syngliocladium cleoni* (Wize) Petch (1925), which, according to Petch always gives trouble whenever wireworms are being bred for research or experimental purposes. A few larvae were found in the field, badly infected with these two fungi. The imperfect stage of both fungi is capable of killing *Corymbites cupreus* larvae, which after death become stiff, 'calcified' in appearance, and extremely brittle. The body tissues are thickly ramified by hyphae, the whole forming a sclerotic mass. A secondary bacterial infection often occurs.

Experiments on the possibility of controlling the wireworm of *C. cupreus* by utilizing these fungi, were made in 1942. A culture medium composed of potato-dextrose-agar was inoculated with the spores. Cultures of each fungus were thus readily obtained and earthenware pots, $3\frac{1}{2}$ in. diameter, filled with soil were heavily impregnated with the fungi. All these pots were kept under conditions as near as possible to those occurring in the field, and varying numbers of larvae were placed in the different series of pots. One series, each with six larvae, contained, besides an artificially produced fungal infection of the soil with the green muscardine, a larva which had recently been killed by this fungus. Not one larva in any of the series of pots was attacked by either fungus even when as many as twelve larvae were kept in one pot and the soil kept abnormally moist. An attack from these fungi seems to depend on some physiological condition of the larvae themselves which, according to some authors, is induced or favoured by overcrowding. Definite evidence on this point is not available, and despite all attempts, none was obtained during the present investigations. All larvae found attacked by the fungi were in their last larval instar and in a sluggish state in preparation for pupation. It is possible that the fungi occur in most types of soil, but that the larvae are not susceptible to attack, except at a certain period, or periods, in their life history when resistance to infection is at a low level. Effective control of *C. cupreus* F. by fungal infections does not appear to be practicable.

Masaitis (1930) correlates the occurrence of fungal infection with parasitism of wireworms by a mite of the genus *Tyroglyphus*. He states that the larvae are first parasitized by the mites which make fine punctures in the chitin of the larva and

fungal spores enter the body through these minute holes. During the present investigations, fungal attacks were not associated with the presence of mites. Numerous dead larvae recovered from the soil both in the field and breeding pots were found to harbour mites belonging to the family Tarsonomidae. In all cases the larvae had died in an extended condition, and on dissection two mites in the hypopus stage were discovered in the fat bodies directly beneath the chitin in the mesothoracic segment of one of them.

Laboratory observations

(*a*) *Experiments on economic importance.* Boxes, each 7 in. deep and with a surface area of 144 sq.in., were filled to within 1 in. of the top with soil in a satisfactory state of fertility. Artificial manure, consisting of $1\frac{1}{2}$ parts of sulphate of ammonia, $3\frac{1}{2}$ parts of superphosphate and 2 parts of muriate of potash, at the rate of 5 cwt. per acre, was added to all the boxes a few days before sowing. Each box contained a population of wireworms equivalent to 3,000,000 per acre, and the tests with both wheat and oats were carried out in triplicate three times a year from 1941 to 1945. In the case of both cereals, eighteen grains (seeds) were sown in each box and the watering was done in such a way as to approach as closely as possible field conditions. The results of these tests are summarized in Table 1.

Table 1. *Relative amount of injury inflicted by a high population of larvae of* Corymbites cupreus, Agriotes *spp. and* Athous niger *to winter wheat and oats*

Crop	Species of wireworm	Grain (seeds) destroyed (%)	Seedlings destroyed (%)	Seedlings survived (%)
Oats	*Corymbites cupreus*	86	9	5
Oats	*Agriotes* spp.	66	8	26
Oats	*Athous niger*	20	1	79
Oats	Nil	0	0	100
Wheat	*Corymbites cupreus*	87	7	6
Wheat	*Agriotes* spp.	68	3	29
Wheat	*Athous niger*	22	5	73
Wheat	Nil	0	0	100

Of the three species of wireworms, *Corymbites cupreus* caused the most injury, and *Athous niger* the least.

In order to determine the relative degree of damage inflicted to wheat, oats and barley by different wireworm populations of *Corymbites cupreus* and *Agriotes* spp., similar series of tests were set up. The experiments were made in triplicate during each season from 1941 to 1945 inclusive. The results are presented, as average figures over the 5 years, in Tables 2–4.

Experiments were also made on the comparative amount of damage done by wireworms of *Corymbites cupreus*, *Agriotes* spp. and *Athous niger* to potatoes. A population of large wireworms equivalent to 1,000,000 per acre was added to the various boxes

TABLE 2. *Relative amount of damage caused by different population levels of larvae of* Corymbites cupreus *and* Agriotes *spp. to wheat*

Population of wireworms per acre	Species of wireworm	Grain destroyed (%)	Seedlings destroyed (%)	Seedlings survived (%)
250,000	*Corymbites cupreus*	24	6	70
250,000	*Agriotes* spp.	9	4	87
600,000	*Corymbites cupreus*	33	21	46
600,000	*Agriotes* spp.	14	16	70
850,000	*Corymbites cupreus*	58	17	25
850,000	*Agriotes* spp.	22	14	64
1,000,000	*Corymbites cupreus*	61	23	16
1,000,000	*Agriotes* spp.	29	24	47
Nil	Nil	0	0	98

TABLE 3. *Relative amount of damage caused by different population levels of larvae of* Corymbites cupreus *and* Agriotes *spp. to oats*

Population of wireworms per acre	Species of wireworm	Grain destroyed (%)	Seedlings destroyed (%)	Seedlings survived (%)
250,000	*Corymbites cupreus*	26	9	65
250,000	*Agriotes* spp.	8	3	89
600,000	*Corymbites cupreus*	31	19	50
600,000	*Agriotes* spp.	15	14	71
850,000	*Corymbites cupreus*	55	19	26
850,000	*Agriotes* spp.	24	16	60
1,000,000	*Corymbites cupreus*	63	22	15
1,000,000	*Agriotes* spp.	34	20	46
Nil	Nil	0	0	96

TABLE 4. *Relative amount of damage caused by different population levels of larvae of* Corymbites cupreus *and* Agriotes *spp. to barley*

Population of wireworms per acre	Species of wireworm	Grain destroyed (%)	Seedlings destroyed (%)	Seedlings survived (%)
250,000	*Corymbites cupreus*	20	4	76
250,000	*Agriotes* spp.	6	4	90
600,000	*Corymbites cupreus*	27	17	56
600,000	*Agriotes* spp.	10	10	80
850,000	*Corymbites cupreus*	43	17	40
850,000	*Agriotes* spp.	18	4	78
1,000,000	*Corymbites cupreus*	43	27	30
1,000,000	*Agriotes* spp.	16	19	65
Nil	Nil	0	0	100

which had been prepared as already described for the other experiments. One unsprouted potato of an average 'seed' size was planted in each box. When the haulms had completely died down, all the tubers were carefully lifted and examined. The results are given in Table 5.

TABLE 5. *Relative amount of damage caused by a high population (equivalent to 1,000,000 per acre) of larvae of* Corymbites cupreus, Agriotes *spp. and* Athous niger *to potatoes*

Index of box	Species of wireworm	No. of holes per tuber	No. of larvae embedded per tuber	Stem injury
1	*Corymbites cupreus*	4	2	0
2	*C. cupreus*	3	3	0
3	*C. cupreus*	5	3	1
4	*C. cupreus*	5	3	0
5	*C. cupreus*	6	4	0
6	*C. cupreus*	4	3	0
7	*Agriotes* spp.	2	1	0
8	*Agriotes* spp.	3	2	0
9	*Agriotes* spp.	3	1	0
10	*Agriotes* spp.	3	1	0
11	*Agriotes* spp.	2	2	0
12	*Agriotes* spp.	3	1	0
13	*Athous niger*	1	0	0
14	*A. niger*	0	0	0
15	*A. niger*	0	0	0
16	*A. niger*	0	0	0
17	*A. niger*	1	0	0
18	*A. niger*	1	0	0
19	Control	0	0	0
20	(None added)	0	0	0
21	(None added)	0	0	0
22	(None added)	0	0	0
23	(None added)	0	0	0
24	(None added)	0	0	0

In no case did the wireworms prevent the tubers from sprouting and only in one instance was the shoot itself attacked, the species involved being *Corymbites cupreus*.

(*b*) *Other laboratory observations.* The larvae of *C. cupreus* are able to move freely along all kinds of surfaces, both rough and smooth, including grass. When walking, their legs display a rhythmic movement, and use is made of the anal pseudopod for balancing purposes. They always move away very rapidly from all sources of light, preferably, if at all possible, by burying themselves in the medium beneath them. One of the first characteristics noted when the larvae were brought to the laboratory was their highly carnivorus habit. When twelve larvae were placed in an earthenware pot containing soil and a potato for food, it was found 7 days later that two larvae had been killed and the softer tissues hollowed out, the only remains being the head capsule, legs and body cuticle. The site of attack is usually the ventral surface of the meso- or metathoracic segments and a larva was frequently discovered having eaten its way into another one until only the posterior portion of its own body remained outside the corpse. This cannibalism was seldom observed in the field, and laboratory investigations showed that this tendency is encouraged by overcrowding.

The larvae of *C. cupreus* are much more ferocious than those of the genus *Agriotes*. For example, when larvae of these two genera were kept in large numbers in flower

pots full of soil, 70% of *Agriotes* spp. had been killed compared with 10% of *Corymbites cupreus*. *C. cupreus* larvae are rather more susceptible to certain conditions than *Agriotes* spp., for instance, larvae of *C. cupreus* kept in dry tubes in the laboratory died within 7 hr., while those of *Agriotes* spp. under identical circumstances, lived at least 12 hr. Comparative data were also obtained on the effect of dryness on them when the two types of larvae were placed in pots of soil which were allowed to become air-dried at ordinary room temperature. At the end of the 22nd day, all the *Agriotes* larvae were still alive, while all the *Corymbites* larvae had died by the 17th day. Again, when they were kept in soil which was constantly retained in a waterlogged condition, all the *Agriotes* larvae survived at least 16 days, but every *Corymbites* larva was dead by the 12th day.

These observations are somewhat surprising, as *C. cupreus* thrives best at high altitudes, where climatic conditions are more rigorous than those at lower levels. No doubt, other factors or combination of factors are involved in the field. Wireworms, in any case, are capable of burrowing deeply into the soil and often do so in order to avoid desiccation. Larvae of the genus *Agriotes* have been recovered down to a depth of 23 in. at two centres in Glamorgan during long spells of dry weather in the summer and those of *Corymbites cupreus* at 19 in. under similar conditions.

Some investigators, including Beling (1883), state that the larvae of the Elateridae are as a whole omnivorous and feed, according to circumstances, on their own larvae and pupae, on smaller soil animals, on vegetable matter and, in the absence of other food, on the humus of the soil or even on the soil itself. Horst (1922) quotes Ritzema-Bos as having seen Elaterid larvae consuming fly maggots, caterpillars and a dead snail. Xambeau (1912) mentions that the larvae of *C. cupreus* feed on the larvae of the genus *Aphodius* in the field, but Horst (1922), on the other hand, thinks that such cases only occur in captivity, when hunger forces the wireworms to tackle animal food. The natural food of *Corymbites cupreus* is vegetable in character, although on rare occasions a larva was seen in the field in the act of burrowing into the body and feeding on the tender tissues of another wireworm or earthworm, which it had presumably killed. Three- or four-year-old larvae of *C. cupreus*, which were kept in the laboratory in soil containing neither vegetable nor animal food, lived through the winter of 1942–3 and appeared quite normal after 7 months of such treatment and moulted at the usual time, the following spring. Taking these results by themselves it seems, therefore, that they can feed and survive on the humus in the soil, but more critical experiments must be made before a definite conclusion can be reached.

Field observations on its economic importance

The larvae of *C. cupreus* were recorded attacking various crops grown on a commercial scale in South Wales and Monmouthshire. The crops suffering the most damage included potatoes, carrots, oats, wheat, barley, rye, swedes, turnips, beetroot, lettuce, tomatoes and brassicae. The burrows in the potato tubers are larger

and more extensive than those made by larvae of the genus *Agriotes*, and the tunnels are reddish brown to black in colour. Potato stems are often seen badly attacked, and in such cases the larva burrows upwards, resulting in complete collapse of the haulm. The damage to carrots is similar to that caused to potato tubers, while cereals are attacked before and after germination. The seed itself is often rendered useless soon after sowing and before germination by the larvae eating their way into the grain and hollowing out the kernel. Seeds of cereals are frequently recovered from the soil with the wireworms embedded in them, only the posterior part of the body being apparent outside. The most severe injury to cereals is inflicted when the seedlings are in a very early stage of growth, but the attack often continues until the plants have become established. When attacked at an early period, the seedling has no power of recovery and the entire shoot can be readily separated from the root system by a gentle pull. After tillering, the plants quickly build up a high degree of resistance to attack, but even at this late stage larvae frequently destroy one or more tillers and are often seen partly embedded in the plant tissue or tunnelling upwards in the stem.

It was found difficult to assess the relative amount of damage done by different population levels of larvae of *Corymbites cupreus* under field conditions, as wherever wireworms of this species occurred, those of the genus *Agriotes* were also present. A field at Gelligaer, Glamorgan, contained a mixed population of 325,000 *Corymbites cupreus* and 300,000 *Agriotes* spp. per acre. The field was 260 yd. long, 160 yd. wide and planted with potatoes. Damage was assessed by digging and examining five sets of samples, each of ten successive plants. The results are summarized in Table 6.

Table 6. *Damage to potatoes in a field in Glamorgan from a mixed population of* 325,000 Corymbites cupreus *and* 300,000 Agriotes *spp. per acre*

Sample units	Tubers attacked (%)			Stems attacked (%)	Tubers with wireworms embedded in them (%)	
	Severe	Slight	Free		*Corymbites cupreus*	*Agriotes* spp.
1	48	44	8	26	29	0
2	56	38	6	12	15	8
3	75	25	0	21	28	6
4	68	20	12	17	21	0
5	61	29	10	14	19	2

These results indicate that 92·8% of the tubers examined were attacked by wireworms, of which 61·6% showed tubers badly riddled, 31·2% slightly damaged; 18·0% of the potato stems had been tunnelled. Further, 29·2% of the tubers contained at least one wireworm embedded in their tissues, 3·2% of them with wireworms of the genus *Agriotes* and 26·0% with those of *Corymbites cupreus*. It would seem that in fields containing about equal populations of *Agriotes* spp. and *C. cupreus*, the latter is the more serious pest to potato crops.

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OBSERVATIONS ON THE BIOLOGY AND CONTROL OF CABBAGE ROOT FLY, *ERIOISCHIA BRASSICAE* (BCHÉ)

By MARY MILES

Wye College, University of London

In the south-east of England egg-laying by *Erioischia brassicae* Bché began in the third week of April and was at its maximum in the fourth week of April and the first week of May. From then on to early October small numbers of eggs were continually present about brassica crops. Egg counts on cauliflowers at the end of April showed up to 141 eggs/plant and on spring cabbage on 6 May up to 117 eggs; the flies showed no preference for newly set plants. Eggs on the soil were almost exclusively those of *Erioischia brassicae*; eggs on the plants were mainly those of *Pegohylemyia fugax* Meig., with small numbers of *Erioischia brassicae* and *Delia cilicrura* Rond.

Larvae were found continuously from the fourth week of April to mid-January, those found in December and January were from the previous autumn. Larvae, up to eighty-one per plant, at the roots of brassica crops were almost exclusively *Erioischia brassicae*. A few *Pegohylemyia fugax* and *Delia cilicrura* occurred, but no *Erioischia floralis* Fall. were found. A technique for separating larvae from the soil and plant roots is described.

Great variation in the size of puparia was noted. Small puparia (4–4·5 mm. long) were heavily parasitized by cynipid wasps.

In trials on cauliflowers and brussels sprouts the efficiency of calomel dust (4%) and benzene hexachloride (flea beetle and wireworm dusts) was compared. An application of B.H.C. wireworm dust at 40 lb./acre (approximately 11,000 plants) appeared to have the same efficiency as an application of calomel dust at the rate of 80 lb./acre. The concentration of benzene hexachloride in flea beetle dust applied at rates up to 40 lb./acre (11,000 plants) was insufficient to secure the measure of control given by calomel dust at 80 lb./acre. In 1949 calomel dust remained effective from 2 July, when it was applied, until 30 August when observations ceased.

Good growing conditions masked injury and were very important in preventing loss from injury by cabbage root fly.

At Wye early cauliflowers, which are grown in 'soil pots' in autumn and wintered in cold frames, are set out in the 'pots' as soon as weather conditions permit. In 1948 planting was finished by 16 March and in 1949 by 19 March. The crop is exposed to attack by the spring generation of cabbage root flies, and while some commercial growers maintain that established plants whose roots are undisturbed at planting are able to withstand attack, others take the precaution of using calomel dust (4%) at the time of planting. Brassica seed crops such as cauliflowers and brussels sprouts have a long life and are exposed to attack by several generations of the flies, and little information on intensity of attack at various seasons is available to assist in determining when control measures should be applied.

The value of 4% mercurous chloride (calomel) dust against cabbage root fly

attack has been demonstrated by Wright (1938, 1940), and Booer (1944) has shown that calomel decomposes slowly into metallic mercury. Timing of applications of calomel dust should be related to the periods of greatest activity of the flies; and the general recommendation that calomel dust be applied within 4 days of setting out susceptible crops needs some modification in the case of early cauliflowers set out in mid-March but not exposed to attack until the flies are on the wing in late April and early May (Min. Agric. Adv. Leaflet, no. 18). If, however, a synthetic insecticide such as benzene hexachloride proved satisfactory, its stability in the soil (Smith, 1948) would make timing of relatively little importance.

Period and intensity of egg-laying

In 1948 and 1949 early cauliflower crops were kept under observation for the presence of eggs, which can be readily seen in the soil with the naked eye. In 1948 the first eggs were seen on 14 April, and by 21 April eggs were numerous about all the plants examined. In 1949 eggs were first found on 20 April and by 25 April large numbers were present.

In 1949 counts were made of the numbers of eggs per plant. On 30 April, when hatching had already begun, five cauliflower plants were examined. The plants were not a random selection, but were chosen from 'blind' and therefore valueless plants at widely separated points in a long narrow bed. The heads were cut off 2–3 in. above soil level and, using a $2\frac{1}{2}$ in. core borer, the soil about the collar and the remainder of the plant were lifted. Eggs were separated from the soil by a flotation method and counted; the numbers found were 106, 114, 86, 138 and 141.

These numbers did not represent the complete egg population about the cauliflowers examined, for at the time of examination eggs were already hatching and empty egg-shells were blown away when the soil was handled. Small numbers of eggs were also found in the shelter of broken leaves, weeds and small clods at a distance from the plant greater than the radius of the core borer, and these were ignored. Further, the soil samples examined contained a quantity of peat from the compost 'pots' and this made complete extraction difficult. It is thought that only 80–90% of the egg population was recorded.

Spring cabbages were also examined. The plants had been set out in October 1948 and were ready for market on 6 May when egg counts were made. Five cabbage plants were selected at random from various points in a large block covering about 2 acres. The head of each was cut off and the root with the soil immediately surrounding it was lifted. Because the stems were thick and tended to be twisted it was not possible to use the core borer, but an attempt was made to lift the soil from a radius of about 2 in. round the plant. The eggs were washed from the stem and soil; the numbers found were 117, 85, 69, 107 and 28 respectively.

Complete extraction was not possible, and since hatching had been in progress for a week many empty chorions were lost. No attempt was made to assess the numbers

of eggs on the aerial parts of the plants though they occurred on the backs of basal leaves, on the petioles and in the leaf axils. The cabbages had not been numbered at the time of lifting, so it was not possible to establish a relationship between the number of eggs about the plant and its position in the crop.

No more egg counts were made, but small samples of soil from the collars were examined for eggs at various times during the season. Composite samples were taken with the tip of a trowel from the bases of a random series of plants and stirred in a trough of water. Floating eggs were collected and tested for viability. This form of qualitative sampling was done during May, June and July and showed that viable eggs were continually present, but not in large numbers as in the last week of April and the first week of May. Sampling for eggs was discontinued after July but sampling for larvae showed that first instar larvae were present throughout August. Females captured in September laid eggs, and in both 1948 and 1949 females taken during the first week of October laid eggs within a few days of capture.

Sampling for eggs through the summer months did not reveal subsidiary peaks of egg production that could be associated with the emergence of flies of the second and third generations. The flies were active throughout the period April–October, and after the middle of May the low egg population per plant may have been the result of the progressive increase in the number of brassica plants.

Species of eggs present

In the course of egg counts some note was taken of the species present. Little comparative work has been done on the eggs of anthomyid species, but during the present studies of British anthomyids it has been found possible to distinguish the eggs of species by their size, colour and surface sculpturing.

Eggs of *Erioischia brassicae* have been adequately illustrated in the literature of this species. Specific characters appear to be the white colour, the well-marked, slightly broken longitudinal ridges, and the pair of thick ribs on the inner, slightly concave surface, which extend from the micropyle ring to the rounded tip where they meet and enclose a more finely reticulated area, through which the larva escapes.

Eggs found on the soil round various brassica crops were almost exclusively those of *E. brassicae*. Large ivory-coloured eggs with coarse, pustulated ridges and three ribs on the inner surface were common but not numerous. Larvae from these eggs hatched but were not reared to maturity, but their habits and the character of the bucco-pharyngeal armature showed them to be predatory.

Eggs of *Pegohylemyia fugax* Meig. and *Delia cilicrura* Rond. were found with eggs of *Erioischia brassicae* in the leaf axils, and on the leaves, petioles and stems of cauliflowers. The eggs of the three species can be readily distinguished by their surface sculpturing. *Pegohylemyia fugax* was by far the most numerous about the aerial parts of the plant, but the records of larvae of *Erioischia brassicae* in the

growing points of turnips and cauliflowers, in the inflorescence of cauliflowers, in the mid-ribs and larger leaf veins of cabbages and cauliflowers, and in the hearts of brussels sprouts support the observation that the eggs are not always laid on the soil.

Observations on the larvae

On hatching larvae of cabbage root fly usually entered the soil and fed in the root mass and in growth cracks in the main stem; later they tunnelled in the cortex and penetrated into the root tissue. They were present at the roots of cauliflowers and cabbages by the 4th week of April and were found on other brassicas during summer, autumn and winter until mid-January of the following year when frost prevented further searching. In 1948 larvae that hatched on 26 April became puparia on 16 May, and on 17 May new, pale puparia were found at the roots of spring cabbages. In 1949 mature larvae were found in radishes on 14 May, and from mid-May onwards during summer and early autumn eggs, first, second and third instar larvae and puparia were present together. First instar larvae were not found after October and only third instar larvae were taken in December and January. Larvae were not found at the roots of winter cabbages in February though numbers of puparia were present, and it appeared that larvae present in January had pupated.

The effect on larvae of flooding their feeding sites was often seen. Submerged larvae tried to escape from waterlogged soil by climbing to the surface. Immersion in water for 2 days failed to drown them.

Numbers of larvae present

In early June, after cauliflowers were harvested, an attempt was made to count larvae present among the roots. The soil was cut round the plant about 4 in. from the stem which was then lifted with the main root mass and the soil adhering to it. The soil was detached from the roots with the fingers, crumbled to release puparia, placed in a glass trough, covered with water, stirred thoroughly and allowed to settle. The root was then washed into a second glass trough in which the washings were allowed to settle; then it was immersed in a large beaker of water for about 1 hr. After soaking, it was thoroughly shaken in the water to release larvae entangled in the roots.

After about 3 hr., when the soil surface was clearly visible through the water, the troughs were examined. Puparia floated and were collected from the sides. Larvae crawled to the surface of the soil in their efforts to escape and lay fully extended. When the soil was fine and made a smooth, even surface, first instar larvae (2 mm. long) could be seen, but all larvae tended to be more difficult to find on a broken surface. The cauliflowers had been set out in soil 'pots' containing peat, consequently peat fragments floated in the water or lay on the surface of the soil, and it was necessary to lift them carefully with forceps to find larvae lying beneath.

Washing the roots in water detached first instar larvae and others feeding superficially, and immersing them in water caused larvae in the root tissue to leave their tunnels and fall to the bottom.

Four cauliflower plants examined on 1–2 June had 74, 46, 81 and 70 larvae and puparia respectively.

Species present

The great majority of the larvae were of the *E. brassicae* type. Predatory larvae of anthomyids and leptids were common. Larvae of *Pegohylemyia fugax* were found in association with *Erioischia brassicae* in the inflorescence of rejected cauliflowers, about the stems and residual leaves of cabbages and cauliflowers, and in brussels sprouts. Small numbers of larvae of *Delia cilicrura* Rond. were found with *Erioischia brassicae* among the roots.

No specimens of *E. floralis* Fall. were observed. From the work of Dr G. Morrison (1937), and from specimens from Scotland that he gave to Prof. H. W. Miles, it was apparent that larvae of *floralis* and *brassicae* could be distinguished by differences in their tubercle pattern. *E. floralis*, a univoltine species often recorded from Scandinavia, Germany, Russia and Japan, does not seem to occur in southern England, and Balachowsky & Mésnil (1935) were not familiar with it in France.

In the course of this work few larvae were reared to maturity and it is not known if *E. pilipyga* Vill., a species apparently closely related to *E. brassicae*, was present. Larvae examined showed a good deal of individual variation in the tubercle pattern, and often malformation and asymmetry, but no evidence of the presence of species other than *E. brassicae* was collected.

OBSERVATIONS ON THE PUPARIA

Puparia examined were 4–7 mm. long, a range so great that it seemed to require investigation. In February 1949 a collection of ninety-six puparia from the roots of winter cabbages were separated into eighty large puparia of 5 mm. and over, and sixteen small puparia of 4–4·5 mm. From the large puparia cabbage root flies and staphylinid beetles (*Aleochara* sp.) emerged, but no record of the numbers of each was kept. From the small puparia a small cabbage root fly (♂) and eleven cynipid wasps emerged. It appeared that the presence of an internal parasite was an important factor affecting the size of the puparium, and that small puparia were usually those formed by larvae whose growth had been checked by parasitism. Since staphylinid beetles feed as external parasites on the pupae (Wadsworth, 1915) they do not interfere with larval development and are, therefore, present in full-sized puparia.

Examination of empty puparia gave information about their previous occupants. Emerging flies split the anterior end of puparia, the opening extending a short distance along each side. Staphylinid beetles and cynipid wasps cut off the anterior end of puparia leaving irregular, serrated edges.

Observations on the adults

Few observations were made on the adults. In 1948 and 1949 they were taken on *Brassica* spp., lettuce and spinach, weeds and the edges of ley fields, on heaps of rakings, and on bare soil from early April to early October. Captured flies lived for some time when fed on dilute sugar solution and they laid eggs, but no attempt was made to observe the length of life and the numbers of eggs laid. There seemed to be at least three overlapping generations, since flies of the second generation emerged during the first week of June.

Control measures

Trials on early cauliflowers

On a variety trial consisting of six randomized blocks (720 plants/block) of cauliflowers treatments with calomel dust (4%) and benzene hexachloride (B.H.C.) in the form of flea beetle dust were compared. No untreated plots could be arranged.

Dust was applied with a knapsack duster on 25 April when egg-laying was at its peak and large numbers of eggs were present. A single puff was directed at the base of each plant and the dust could be clearly seen covering an area of approximately 6 in. square around the stem of each plant. The rates of application per acre of approximately 11,000 plants at a 2 ft. square spacing were 80 lb. calomel dust and 17 lb. flea beetle dust. The amount of flea beetle dust applied was less than had been intended but the soil around the plants was covered so no second application was made.

No observations on the degree of infestation could be made until after the cauliflowers were cut. Then counts were made on twelve plants from the plots treated with calomel and flea beetle dust. The method already described (p. 263) was used to extract larvae and puparia. Available apparatus limited the number of plants that could be examined at one time, and consequently counts were made over a week. The effect of the interval between counts was probably negligible because the emergence of the second generation of flies was just starting.

Table 1. *Numbers of larvae and puparia at the roots of cauliflower plants*

Date of examination	Calomel dust (4%)		Flea beetle dust	
	Plant 1	Plant 2	Plant 1	Plant 2
1 June	2	4	66	21
2 June	0	17	32	38
3 June	3	3	37	14
6 June	11	0	35	43
			46	56
7 June	4	8	49	52
	11	15		
Mean	6·5		40·8	

Table 1 shows that at the rates used calomel dust was more effective than flea beetle dust (B.H.C.) in controlling cabbage root fly. The growing plants showed no ill

effects of attack, and statistical examination of the weights of cauliflowers harvested gave no significant differences that could be associated with intensity of maggot attack at the roots. It appeared, therefore, that, under the conditions at Wye, injury by cabbage root fly was masked by the cultural practice of setting out the plants in heavily manured soil without disturbing their roots, and by supplying water by means of an irrigation system to prevent wilting and encourage rapid growth.

Trials on brussels sprouts

Brussels sprouts were set out on 14 June at a spacing of 1 ft. in three rows in order to give information on infestation by cabbage root fly during the summer months, and to compare the protective value of applications of calomel (4%) and two commercial dusts containing benzene hexachloride: flea beetle dust and wireworm dust. Frequent observations were made on the egg population so that the application of the dusts could be timed to correspond with the peak of egg-laying by the second generation of flies. Examination of trowel-tip soil samples showed eggs to be present continuously but the egg population remained low. The plants were dusted on 2 July, and by increasing the number of puffs per plant the rate of

TABLE 2. *Numbers of larvae and puparia at the roots of brussels sprouts*

Date of examination	Calomel (4%)		Flea beetle dust		Wireworm dust	
	Plant 1	Plant 2	Plant 1	Plant 2	Plant 1	Plant 2
20 July	8	7	8	12	0	1
20 July	5	27	11	6	4	1
21 July	5	5	38	12	4	14
22 July	1	2	12	6	10	6
22 July	1	0	12	4	0	0
25 July	0	1	9	2	0	2
25 July	0	7	14	18	11	10
27 July	8	0	18	34	4	3
27 July	0	2	17	10	1	1
29 July	8	4	16	33	3	2
2 August	15	2	12	14	4	8
3 August	8	0	23	23	5	9
4 August	3	2	23	17	1	0
6 August	0	1	2	0	10	0
8 August	1	1	5	14	1	1
9 August	5	2	26	23	4	1
10 August	9	9	28	17	9	9
11 August	6	11	24	15	4	11
12 August	6	1	11	3	5	25
15 August	5	0	19	11	5	8
16 August	1	7	6	4	5	3
17 August	4	0	10	2	13	4
23 August	14	3	15	5	2	9
24 August	1	0	8	16	0	5
25 August	1	3	11	6	5	5
30 August	0	4	12	7	0	4
Mean	4·1		13·5		4·8	

application of B.H.C. dusts was increased to approximately 40 lb./acre of 11,000 plants, and calomel dust was again applied at approximately 80 lb./acre.

The selection of plants for examination was not at random. The rows were gradually thinned by removing alternate plants so that a stand of plants 2 ft. apart was left; and those examined on any given date were in approximately corresponding stations in the rows. The data on infestation collected during July and August are shown in Table 2.

The examination showed that first instar larvae were reaching the plants continuously during July and August. Flea beetle dust at the rate of 40 lb./acre (approximately 11,000 plants) was much less effective against infestation by cabbage root fly than calomel dust at 80 lb./acre. On the other hand, wireworm dust at the rate of 40 lb./acre gave a similar degree of protection to that given by calomel dust. An interesting feature of the data was the evidence of the stability of mercurous chloride (calomel) in the soil. At Wye, in 1949, the calomel dust maintained its efficiency from 2 July, when it was applied, to 30 August, the date of the last observations, and it is probable that a second application, which is often recommended, may be unnecessary. The crop of brussels sprouts, like the crop of early cauliflowers under observation in the spring, showed no ill effects of attack by cabbage root fly.

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THE COMPARISON OF SUCTION TRAP, STICKY TRAP AND TOW-NET FOR THE QUANTITATIVE SAMPLING OF SMALL AIRBORNE INSECTS

By C. G. JOHNSON

Department of Entomology, Rothamsted Experimental Station, Harpenden, Herts

(With 8 Text-figures)

The sticky trap and stationary aerial 'tow-net' catch insects which alight or fly on to them or are blown against them by the wind. It would be expected that such traps would be inefficient in light winds or in calm weather; and even though their efficiency should increase with stronger winds, errors of unknown magnitude may occur not only in estimations of density and of proportions of species in the air, but also with comparisons of actual catches. These errors are due to unknown degrees of weighting as the traps sample by means of a variable wind from a changing population density. The suction trap, on the other hand, samples a constant quantity of air in all relevant wind-speeds and does not appear to suffer so seriously from these disadvantages. It also works efficiently in perfectly calm weather when maximum densities of insects are often in the air.

The performances of the three traps in the field operating over a range of wind-speeds are described. Particular attention has been paid to aphids, for which the sticky trap and tow-net are generally used and for which the suction trap was primarily designed. Density estimates of these insects in winds below about 3 m.p.h. are much larger when calculated from suction trap catches as compared with estimates from sticky trap and tow-net catches. There is reason for the belief that the suction trap is neither attractive nor repellent to aphids to a significant extent, and that it catches these insects at random by virtue of its air stream alone; weighting of catches due to variable quantities of air being sampled does not occur. It is considered, therefore, that the suction-trap values of density are likely to be the more accurate ones. Sampling of other small insects is also discussed.

Introduction

The present study arose from the need for a reliable trapping method for airborne aphids. Two kinds of trap have usually been used for these insects, namely the sticky trap and the stationary aerial 'tow-net' (Broadbent, 1948; Broadbent, Doncaster, Hull & Watson, 1948; Doncaster & Gregory, 1948; Hardy & Milne, 1938; Gorham, 1946; Freeman, 1945; Johnson (unpublished). Another trap, less commonly used, is the rotating net (Williams & Milne, 1935; Thomas & Vevai, 1940) but this is not considered here.

Hardy & Milne and Freeman used tow-nets in their aerobiological studies, and the latter standardized the catch for a certain volume of air passing through the net, thus in fact estimating density. The sticky trap, widely employed in plant virus epidemiology, has not been used, at any rate with aphids, to estimate density. Doncaster &

Gregory (p. 56) stated that: 'The numbers caught on the trap for a given volume of air flowing past must be altered by many factors, but it is likely that the most important factor is the number of aphides present in that volume of air. At present we have no data to show how the number of aphides caught on a trap is related to the number in the air. Physical considerations suggest that at least in a wind all the aphides present in the cross-section subtended by the trap would be deposited involuntarily.' So, in virus work, it is the actual catches in different places which have been used (Broadbent & Gregory, 1948) and the sticky trap has also been used to compare the proportions of different species in the air (Broadbent, 1948).

The actual numbers caught on traps may be a useful index in virus epidemiology, but for work on the quantitative biology of the insects it is the numbers per unit volume of air, or density, rather than the actual numbers caught which is often of more significance. Moreover, the relative efficiency of traps can be gauged only after catches have been standardized for volume of air sampled or, in other words, by means of the density estimates.

Thus when work on aphid ecology and aerobiology was started in this department in 1946 it was considered necessary to gather, in the words of Doncaster & Gregory, 'data to show how the number of aphides caught on a trap is related to the number in the air'.

But both the tow-net and the sticky trap appeared to possess obvious disadvantages; for since they depend so much on the wind for trapping they could not be expected to work efficiently in calm weather when maximum numbers of insects fly. In addition, variation of wind-speed during the trapping period might cause the catch, the relative numbers of different species caught and the density estimates to be weighted to an unknown degree.

To assess the efficiency of a trap is a difficult matter because there is no standard for calibration; for no trap is known which will extract all the insects, or even a known proportion of the total, from a given volume of air. An attempt had to be made, therefore, to overcome two difficulties at the same time and to design a trap which, by working efficiently in calm air and, independently of and unaffected by the wind, would not have the drawbacks of the net and sticky trap; and being neither attractive nor repellent, but by sampling at random from a known volume of air, might hold out some promise for the development of a standard for the assessment of the efficiency of other traps.

With these facts in mind the suction trap was devised; this sucks in air at a constant rate in all relevant wind-speeds, and preliminary experiments indicate that it is neither attractive nor repellent to aphids to a significant extent. It gives much higher density estimates than either net or sticky trap with aphids at low wind-speeds, and since these higher estimates are almost certainly not due to attraction and cannot be attributed to weighting errors they are considered likely to be the more accurate. Nevertheless, in spite of its advantages and possibilities, density estimates made with it are still arbitrary and will remain so until the errors of the trap have been

measured; attempts at this are being made and these include experiments on the degree of attractiveness or repellency of the trap.

Suction traps have been used by entomologists before (see Peterson, 1934), but mainly for catching large samples rather than for making more accurate density estimates. For example, a light was sometimes used and the mouth of the trap directed into the wind (Headlee, 1932). Such features have been avoided in the suction traps described here which aim at random sampling and the elimination of wind effects.

In the following pages some theoretical possibilities of error with sticky traps and nets are discussed; after this is a description of the comparative experiments in the field with the three types of trap.

The terms 'density' and 'efficiency'

The term 'density' is used to express the number of insects per unit volume of air. In practice this is always a mean value of a density which is fluctuating while the sample is being taken. The density estimate has been used here because it is at present the only basis on which the relative efficiencies of traps can be ascertained and because we wish to know the relation of the trap catch to the number of insects in the air.

The term 'efficiency' expresses not the rate of capture but the proportion of total insects in a given volume of air about to be sampled which is caught and retained by the trap. A trap is 100% efficient if it catches all, and 50% efficient if it catches half, the insects in the air about to be sampled.

Assumptions made in the following work

In the theoretical considerations and in the calculations made from field observations described below, it has been assumed that all the traps sample with 100% efficiency. In this case the suction trap catch is the same as the density in the volume of air sampled; with the sticky trap the quantity of air sampled by the trap surface is, for practical purposes, directly proportional to the speed of the wind past it.

With the net, however, the proportion of air which actually passes through, diminishes disproportionately with decrease in wind-speed. This fact has been taken into account in the calculations of density from the field data (see p. 273), but for the simple presentation of the theoretical aspects it has been neglected.

Theoretical possibilities of biased catches and estimates with sticky trap and net

In this section attention is drawn to certain theoretical sources of error; how far they apply in the field is not known.

The aerial density of insects usually changes in inverse order to the wind-speed. If a trap uses the natural, variable wind for trapping, serious errors are possible not

only in the density estimates and in assessments of the proportion of different species in the air, but also in the comparison of the actual numbers caught if these are related to the total run of the wind past the trap or to a mean wind-speed and nothing is known about the variation of wind-speed over the trapping period. These points are illustrated in the hypothetical examples below.

Errors in density estimates

	1st hr.	2nd hr.	
Actual density per mile of air subtended by trap	100	10	Mean density per mile for the 2 hr. = 55
Wind-speed, m.p.h.	1	10	Total wind run for 2 hr. = 11 miles
Calculated catch	100	100	Total catch for 2 hr. = 200
Mean density per mile estimated from 2 hr. catch			200/11 = 18·2

Thus, although the actual mean density is 55, the estimated density is only 18·2.

Errors in total catch

If the mean wind-speed in two places is the same, the catch may be different simply on account of differences in wind-speed variation.

If the density-wind-speed relationship is arranged on an arbitrary, though reasonable scale, and the catch calculated from the formula on p. 272, then at a uniform wind-speed of 1 m.p.h. and a density of 45 insects/1000 cu.ft., 99 insects are caught in 1 hr. If the hour is subdivided into nine equal periods each with a different wind-speed but with a mean speed of 1 m.p.h. for the hour, then the catch is only 81. The following example illustrates this.

Wind-speed, m.p.h. (6·7 min. periods)	Density per 1000 cu.ft.	Catch
0	100	0
0·25	80	4·9
0·50	65	8·0
0·75	54	10·0
1·00	45	11·0
1·25	37	11·3
1·50	32	11·8
1·75	28	12·0
2·00	25	12·2
Mean wind-speed for the hr. = 1·00 m.p.h.		Total catch for the hr. = 81·2

The actual mean density per 1000 cu.ft. over the hour is 51·8; that calculated from the total catch and the mean wind-speed, 36·5.

Actually more insects would be caught in practice at the lower wind-speeds than shown in the example, due to deliberate alighting on the trap, but this does not invalidate the argument. Moreover, lengthy trapping periods in windy places might result in more variability than the example shows.

Errors in estimations of the proportions of different species in the air in two places

	1st hr.	2nd hr.	Total 2 hr.	X/Y
	Actual density and proportions of two species X and Y			
Traps A and B	$1X$ $10Y$	$10X$ $1Y$	$11X$ $11Y$	1·00
	Proportions actually caught by the traps			
Trap A	$1X$ $10Y$	$20X$ $2Y$	$21X$ $12Y$	1·75
Wind-speed	1	2		
Trap B	$2X$ $20Y$	$30X$ $3Y$	$32X$ $23Y$	1·39
Wind-speed	2	3		

Thus the proportions of X and Y in the air over the 2 hr. may be equal, although this would not be shown by the trap catches.

In the above examples only those density changes due to the direct effect of wind on activity have been considered. Changes in density due to other factors might lead to estimates which are too high rather than too low; they may also have a compensating effect on changes due to wind-speed and thus mask a more simple relationship.

The estimation of density in the field

The sticky trap

The sticky trap used was a cylinder of galvanized iron 12 in. long, 5 in. diameter, painted white. Around this was fixed a sheet of stiff cellophane on which a grease-banding preparation was smeared. The trap was identical to that described by Broadbent *et al.* (1948) and by Broadbent (1948) and the data apply only to a trap of this size, shape and colour.

Density estimations with this trap have been made only with respect to wind-speed changes. Turbulence, impaction and momentum have been neglected. It is assumed that if density remains constant the catch varies in direct proportion to the wind-speed and that all insects approaching the trap within its width will be caught and none diverted by the slip-stream around the trap.

Density estimations are calculated as follows: effective trap area presented to the wind = 0·42 sq.ft.; number of insects per cu.ft. of air = D; number of insects caught per hr. = T.

Then the number of insects caught in 1 hr. at a wind-speed of x ft./sec. will be expressed as

$$T = D\,(1512x),$$

from which D for any volume of air may be calculated.

The aerial tow-net

The net was conical, 33 in. in diameter at the mouth, 42 in. long, and was made of hard-spun single thread cotton voile, $1\frac{3}{4}$ oz./sq.yd., 57 warp, 53 weft. It was kept open by a light bamboo hoop to which the rim of the net was attached; a boom running along the top on the outside kept the net stiff. Two swivels at opposite

points on the bamboo hoop allowed the net to rotate round a vertical wire, thus keeping it faced into the wind. Similar nets have been used to study aeroplankton (Hardy & Milne, 1938; Freeman, 1945; Johnson (unpublished)) and net traps working on the same principle have been used by Gorham (1946) for aphids and by other entomologists (see Peterson, 1934).

The air which passes through the net is only a proportion of the amount which would pass through a similar area without the net; the higher the wind-speed, the greater is the proportion which the net allows to pass. The following values have been found experimentally for the net used in the present tests; for a description of the procedure see appendix (p. 285).

Wind-speed (m.p.h.)	Percentage air passing through the net
0·8	25·0
1·1	63·6
4·1	70·7
4·5	80·0
8·4	82·1
8·6	80·2
16·4	88·4
16·7	89·2

Thus, in order to estimate the density with the tow-net not only must the run of the wind during the trapping period be known but a mean value for the wind-speed over the same period calculated; from this a correction for the percentage of air passing through the net must be made from the smooth curve drawn from the above data.

Density estimates from the net are obtained from the following formula:

$$D = (T/(Ax)\,3600)\,K_x/100,$$

where D = no. of insects per cu.ft. of air, T = no. of insects caught per hr., A = area of net opening (i.e. 5·97 sq.ft.), x = mean wind-speed in ft. per sec., K_x = percentage of air passing through the net at a wind-speed of x ft./sec.

The suction trap

Most of the results given here have been obtained with a suction trap having a 9 in. Vent-Axia fan (Johnson, 1950), although for Exp. 1 a 6 in. fan was used. It has been shown that if a wind blows across the mouth of the suction trap there is a reduction in the quantity of air sampled and the reduction is more pronounced the higher the wind-speed. This effect can be eliminated if a flared rim is fitted round the trap opening. Most of the experiments described here were made before the effects of this rim were known and therefore a small correction for the reducing effects of wind have to be applied. For details of this correction see Johnson (1950).

A 9 in. trap samples approximately 19,000 cu.ft. of air per hr. in still air. If p is

the percentage reduction in a certain wind-speed, D the number of insects per cu.ft. of air and T the number caught per hr., then

$$D = T/190p.$$

The 6 in. trap samples approximately 5500 cu.ft. of air per hr., and with its appropriate values of p (Johnson, 1950) the density estimation can similarly be calculated.

The comparative performances of the traps in the field

Exp. 1. *Six-inch suction trap and sticky trap*

This work was done in the autumn and winter of 1947 at a sewage works where the chironomid, *Spaniotoma* (*Orthocladius*) *perennis* Mg. was almost the only insect caught; the small proportion of other insects trapped have not been used.

A 6 in. suction trap, a sticky trap and a cup-anemometer (starting wind 1·8 m.p.h.) were set up 4 ft. apart with the rim of the fan, the middle of the sticky trap and the anemometer 5 ft. above the ground. The sticky material was renewed at 10 to 14-day intervals. Insects were removed from the traps twice a day at approximately 10 to 12 hr. intervals and the data reduced to numbers caught per 10 hr. The run of the wind over the trapping period was taken and the mean wind-speed for the period found. The catches were then arranged in the mean wind-speed groups of 0·5 m.p.h. and the anemometer correction applied to the wind-speed groups.

The actual numbers caught are shown in Table 1 and the density estimations in Fig. 1. Estimates of density from the suction trap catches are between three and seven times as great as those calculated from sticky trap catches. This is particularly noticeable below 5 m.p.h. where numbers are large; at higher wind-speed the differences tend to become of even greater proportions, although no such trend is to be observed if the data are put on a logarithmic scale.

Exp. 2. *Nine-inch suction trap, sticky trap and tow-net*

The three traps were set up together, with a cup-anemometer (starting wind 1·8 m.p.h.) 5 yd. apart at the corners of a square in a field well away from local influences likely to affect individual traps. The centres of the net, sticky trap, the top of the suction trap and the anemometer were 40 in. above ground level. The grease-banding material on the sticky trap was renewed at 10 to 14-day intervals; no obvious change in the ability to catch insects was noticed during this period and this factor did not seriously affect the comparison (see Exps. 3 and 4 where grease banding was renewed every 2 days). Insects were removed from the traps at intervals of 1 hr. The sticky trap was protected by a cover when not in use. The hourly wind-run was taken and the mean wind-speed calculated from it.

All observations of the catch and the mean wind-speeds were placed in 0·5 m.p.h. groups. The mean wind-speed for the group was calculated and the anemometer

TABLE 1. *Mean number of* Spaniotoma perennis *Mg. (Diptera, Chironomidae) caught per 10 hr. with 6 in. suction trap and sticky trap*

Wind-speed (m.p.h.)	10.00–24.00 hr.			22.00–12.00 hr.		
	No. of 10 hr. periods	Mean no. caught per 10 hr. period		No. of 10 hr. periods	Mean no. caught per 10 hr. period	
		Suction	Sticky		Suction	Sticky
1·4–1·8	5	424·1	51·4	11	121·6	17·5
1·8–2·1	8	179·0	45·7	7	31·5	8·0
2·1–2·5	9	153·2	34·0	9	42·3	20·4
2·5–2·9	5	96·2	36·1	9	31·9	11·5
2·9–3·3	10	113·6	23·7	7	30·9	5·5
3·3–3·7	6	33·1	9·7	6	15·1	8·7
3·7–4·1	3	15·5	4·0	3	31·6	13·3
4·1–4·6	2	5·1	9·5	4	5·2	2·0
4·6–5·0	4	39·4	6·2	5	7·5	4·0
5·0–5·5	7	24·9	10·3	5	15·9	7·2
5·5–5·9	3	20·4	4·5	3	4·0	6·2
5·9–6·5	1	1·7	2·6	—	—	—
6·5–6·9	2	1·9	0	2	0	0
6·9–7·4	2	2·1	0·8	—	—	—
7·4–7·9	1	3·6	2·7	1	6·0	3·4
7·9–8·4	1	0	0	1	0	0
8·4–8·9	—	—	—	2	4·6	1·5
12·1	1	4·3	2·6	—	—	—

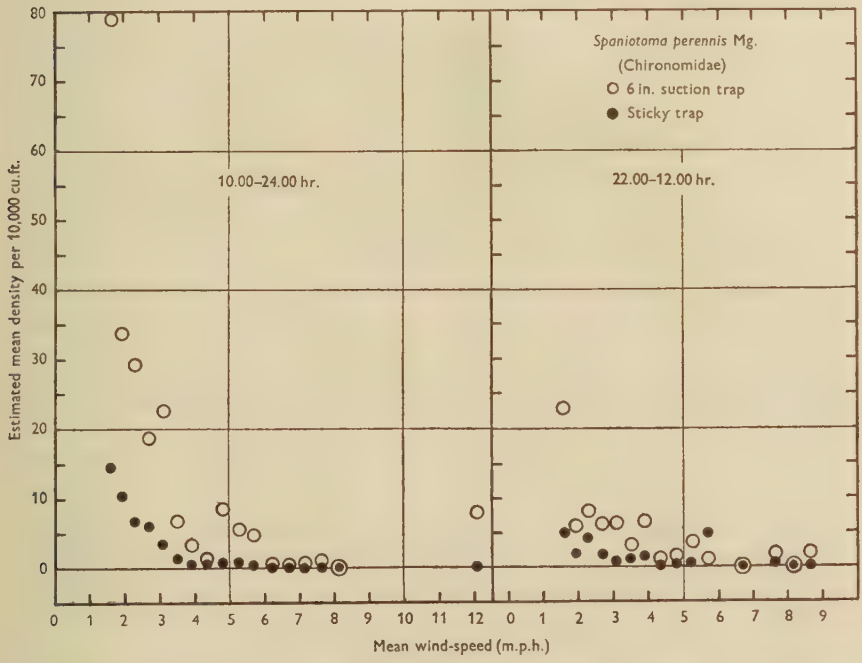

Fig. 1. Mean densities of *Spaniotoma perennis* Mg. (Chironomidae) as estimated by 6 in. suction trap and sticky trap.

correction applied. The catch was arranged in three categories; namely aphids, all other insects less than approximately $\frac{1}{4}$ in. long and those larger than this.

TABLE 2. *Mean numbers of aphids and other insects caught per hr. with 9 in. suction trap, sticky trap and tow-net*

Mean wind-speed (m.p.h.)	No. of 1 hr. periods	Mean nos. caught per hr.								
		Aphids			Others under $\frac{1}{4}$ in.			Others over $\frac{1}{4}$ in.		
		Suction trap	Sticky trap	Tow-net	Suction trap	Sticky trap	Tow-net	Suction trap	Sticky trap	Tow-net
2·0	13	5·38	0·23	0·85	78·9	17·2	3·7	1·54	1·08	0·15
2·3	21	3·24	0·38	0·76	75·0	17·1	5·5	2·86	1·76	0·29
2·6	14	2·14	0·57	1·00	52·4	16·1	7·9	2·14	1·36	0·29
3·0	10	0·40	0·10	0·60	45·9	15·2	12·6	2·20	1·60	0·30
3·6	3	1·33	0	1·67	47·0	25·3	18·3	1·67	2·00	2·00
3·9	7	0·86	0·29	1·14	36·1	23·0	23·1	2·43	3·57	2·00
4·4	3	2·33	1·33	4·33	18·3	9·3	18·0	0·33	0	0
5·0	1	3·00	0	3·00	16·0	3·0	5·0	2·00	0	0
5·7	2	0	0	1·50	10·0	4·5	22·5	0	0·50	0
6·9	4	0	0	0·25	1·5	1·8	19·3	0·25	0	1·25
8·1	2	0	0	1·00	3·5	1·0	27·5	0·50	0	0·50
9·2	3	0	0	1·00	1·3	1·3	39·3	0·33	0·33	1·00
10·3	2	0	0·50	1·00	1·5	2·0	63·0	0·50	0	2·00

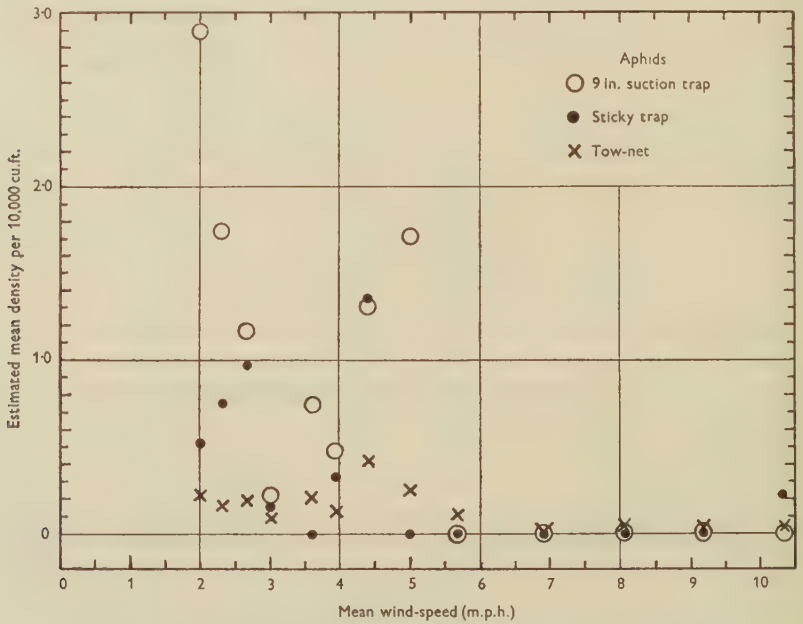

Fig. 2. Mean densities of aphids as estimated by 9 in. suction trap, sticky trap and tow-net (Exp. 2).

Aphids (Table 2, Fig. 2). At wind-speeds less than about 3 m.p.h. density estimates from suction trap catches are much higher than those from the sticky trap (Fig. 2). *t* tests have been made on both the arithmetic mean densities and on the mean log densities. In the two lowest wind-speed groups, 2·0 and 2·3 m.p.h., the differences between the estimates from suction and sticky traps are highly significant; the differences in the next two groups, 2·6 and 3·0 m.p.h., though still showing larger estimates with the suction trap are not statistically significant. It may be, however, that real equality in the estimates is not attained below about 4 m.p.h.

The apparent inefficiency of the net below about 7 m.p.h. is striking and the differences in estimates between the net and the suction trap highly significant. Above this wind-speed the net gave slightly higher density estimates than either of the other two traps; this was due to the virtual absence of aphids from the catches of the latter while an occasional aphid was caught in the net. This was no doubt due to the very much larger volume of air sampled by the net at the higher wind-speed compared with the other traps (Fig. 3).

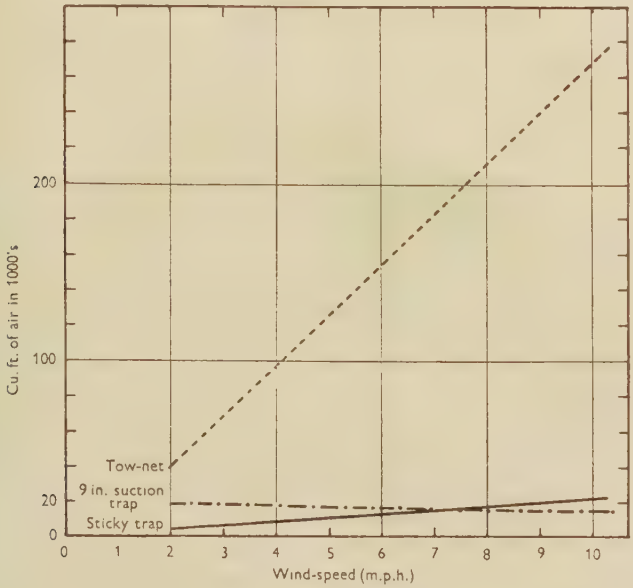

Fig. 3. Calculated quantities of air sampled per hr. by 9 in. suction trap, sticky trap and tow-net.

Insects other than aphids (*Table* 2)

Using logs, no statistically significant differences were found between the density estimates of insects less than about $\frac{1}{4}$ in. long with either suction or sticky trap at

each mean wind-speed over the whole range; neither were the general means above and below 5 m.p.h. significantly different (Fig. 4).

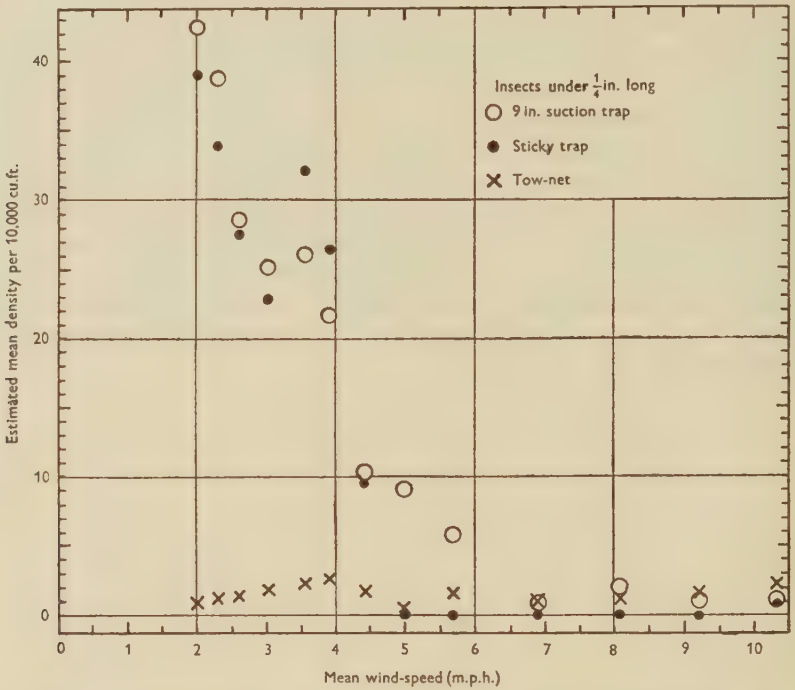

Fig. 4. Mean densities of insects (other than aphids, and less than approximately ¼ in. long) as estimated with 9 in. suction trap, sticky trap and tow-net (Exp. 2).

The densities estimated from the net catches were extraordinarily low, however, and significantly different from those of the other two traps below 4 m.p.h. Above this, none of the three traps gave significantly different estimates either when the mean values for each wind-speed group were considered separately or were combined to give a general mean. It is possible, however, that the net values do not really coincide with those from the other traps until about 7 m.p.h. or even beyond.

With the larger insects (Fig. 5) it is the sticky trap which gives the highest density estimates below 5 m.p.h. Above this none of the traps gives obviously different values.

Exp. 3. Nine-inch suction trap and sticky trap

Two suction and two sticky traps were erected 4 ft. apart at the corners of a square so that similar traps occupied diagonally opposite corners. The height to the rim of each suction trap and to the middle of the sticky traps was 4½ ft. A sensitive cup anemometer (starting wind 0·9 m.p.h.) stood in the centre of the square on a

post at the same height as the traps. Each trap was moved into the place of its neighbour at the end of each hour when the run of the wind was taken and all the traps cleared. The sticky material was renewed every 2 days so that possible ageing effects from which the two previous experiments may have suffered were eliminated.

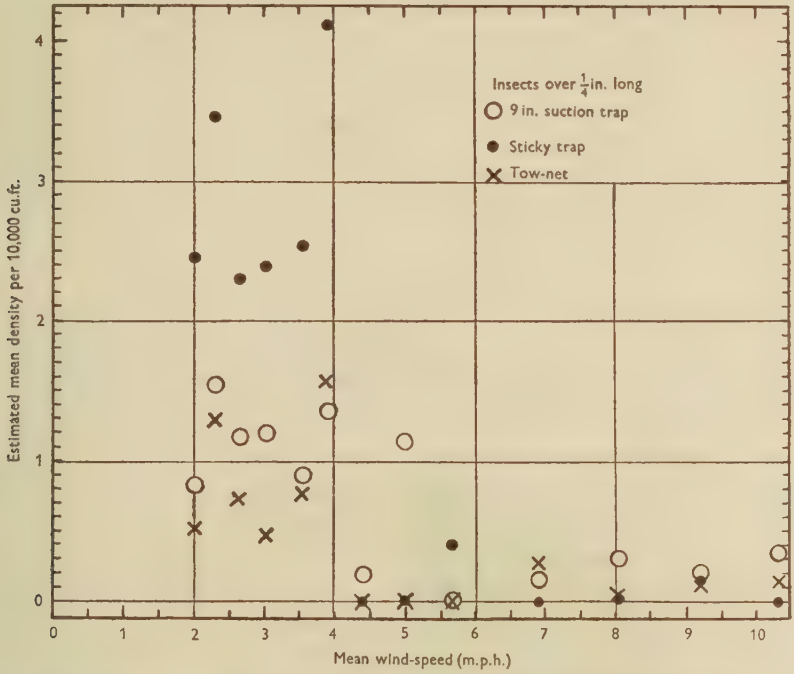

Fig. 5. Mean densities of insects (other than aphids, and more than approximately ¼ in. long) as estimated with 9 in. suction trap, sticky trap and tow-net (Exp. 2).

TABLE 3. *Mean numbers of* Myzocallis coryli (*Goetze*) (*Aphididae*) *caught by the 9 in. suction trap and the sticky trap*

Mean wind-speed (m.p.h.)	No. of 1 hr. periods	Mean no. per trap per hr.	
		Suction	Sticky
Less than 1·0	8	1·25	0
1·0–1·5	50	1·56	0·14
1·5–2·0	48	2·98	0·13
2·0–2·5	10	1·30	0·10

(*a*) This experiment was made in a small sheltered glade surrounded by apple and hazel trees. The only aphid present in suitable quantities was *Myzocallis coryli* (Goetze) which is the only insect considered in this experiment. Table 3 gives the mean number caught per hr. per trap. Fig. 6 shows the density estimates in relation

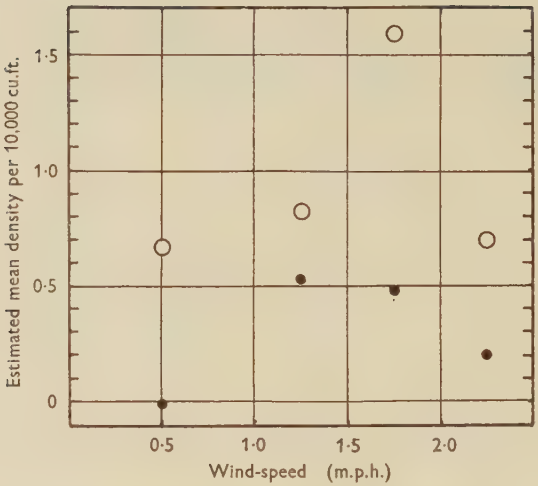

Fig. 6. Mean densities of *Myzocallis coryli* (Goetze) (Aphididae) as estimated with 9 in. suction trap, and sticky trap (Exp. 3*a*). ○, suction; ●, sticky.

to wind-speed. The results are very similar to those with other aphids and chironomids in the previous experiments, and suction traps gave significantly higher density estimates than sticky traps up to 2 m.p.h.

(*b*) The above experiment was repeated in the centre of a large plot of newly dug soil in a walled garden and well away from any local influence likely to affect individual traps. Many species of aphids were on the wing as well as other small insects, particularly *Lachesilla pedicularia* (L.) (Psocoptera). The insects considered have been limited to those less than approximately the size of a house-fly. Aphids, small beetles and the psocopteron have been grouped separately; Diptera and Hymenoptera have been combined because of the frequent difficulty in deciding which was which on the sticky trap; time did not allow them to be cleaned for closer examination.

TABLE 4. *Mean numbers of aphididae and other insects caught per hr. per trap. Exp. 3b: 9 in. suction trap and sticky trap*

Mean wind-speed (m.p.h.)	No. of 1 hr. periods		Mean no. per trap per hr.			
			Suction		Sticky	
	Aphids	Others	Aphids	Others	Aphids	Others
1·0–	20	22	28·65	36·91	1·20	8·50
1·5–	28	28	14·25	40·50	0·46	14·79
2·0–	40	40	15·90	22·75	1·20	7·90
2·5–	24	28	9·25	26·64	1·88	12·89
3·0–	10	14	2·30	8·29	0·50	4·86
3·5–4·0	4		3·25		1·00	

Other insects not considered separately but combined in the total catch were mainly small Homoptera and Heteroptera.

TABLE 5. *Exp. 3b: mean numbers of* Lachesilla pedicularia *(L.) (Psocoptera), Coleoptera and Diptera and Hymenoptera less than approximately* $\frac{1}{4}$ *in. long per trap per hr. caught with 9 in. suction trap and sticky trap*

Mean wind-speed (m.p.h.)	Psocoptera (*L. pedicularia*)		No. of 1 hr. periods	Coleoptera		No. of 1 hr. periods	Diptera and Hymenoptera		No. of 1 hr. periods
	Suction trap	Sticky trap		Suction trap	Sticky trap		Suction trap	Sticky trap	
1·0–1·5	10·46	3·71	24	8·94	0·63	16	17·73	4·00	22
1·5–2·0	14·93	6·46	28	7·29	0·50	24	17·75	7·25	28
2·0–2·5	7·82	2·46	28	1·40	0·53	30	18·33	6·60	30
2·5–3·0	3·33	2·17	6	2·31	0·96	26	16·50	9·88	8
3·5–4·0	—	—	—	—	—	—	4·88	2·50	8

Density estimates made from the catches are shown in Figs. 7 and 8. As in other experiments the estimates for aphids were higher with the suction trap than with the sticky trap below 3 m.p.h. Similar results were obtained with small Coleoptera; but with Diptera and Hymenoptera and with Psocoptera the higher estimates occurred with the sticky traps.

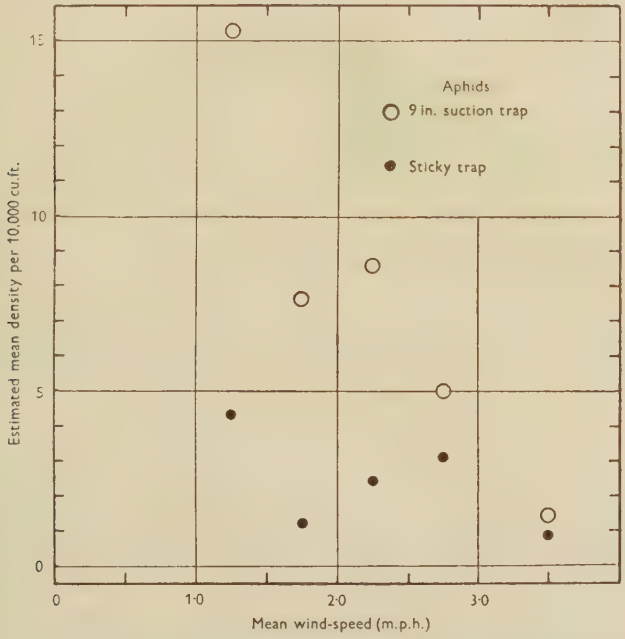

Fig. 7. Mean densities of aphids as estimated by 9 in. suction trap and sticky trap (Exp. 3*b*).

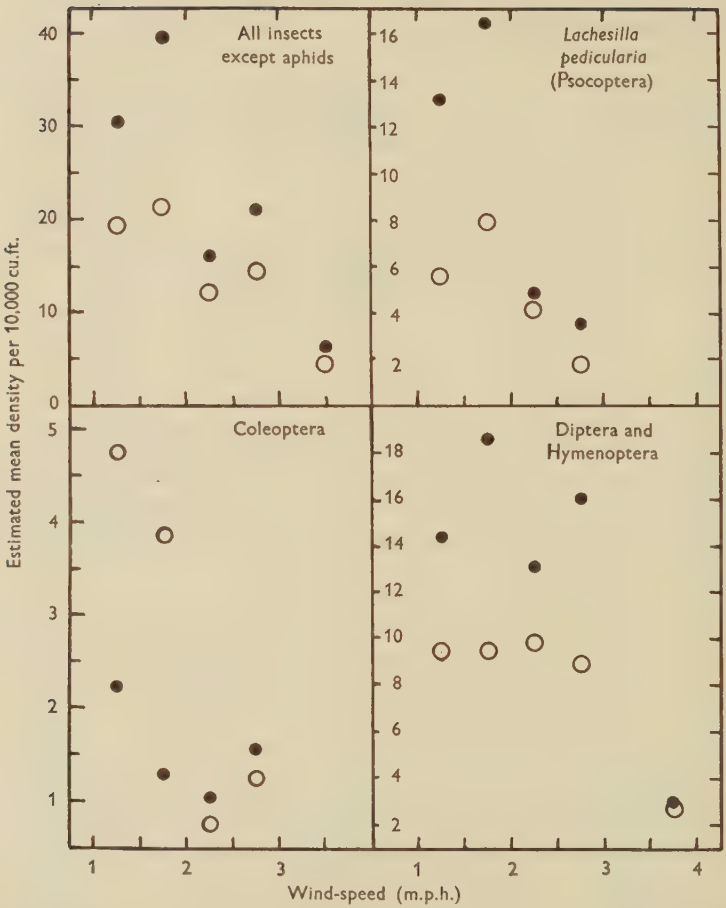

Fig. 8. Mean densities of insects (other than aphids, and less than approximately $\frac{1}{4}$ in. long) as estimated by 9 in. suction trap and sticky trap (Exp. 3*b*). ○, suction; ●, sticky.

Interpretation of field experiments

The use of regressions of density on wind-speed has been avoided with the present results, since it is probable that other factors besides wind-speed affected the populations sampled. But since all traps for comparison were operated simultaneously the relative catches and relative density estimates between the different traps bear comparison between each wind-speed.

With the suction trap the rate of air intake is not seriously affected by wind-speed or is subject to only a relatively small correction. In higher wind-speeds, however, the momentum of the insects has increased and it is possible that a greater pro-

portion may pass over the top of the trap without being captured. In view of the preliminary nature of this work and the smallness of the insects concerned this factor has had to be temporarily neglected.

The size of a sample taken by a trap

For a trap to catch a large number of insects is not always an indication of its superiority; yet it is usually better to have large than small samples and that these should be caught as quickly as possible so as to avoid an undesirable amount of climatic or population change while the sample is being taken.

TABLE 6. *Simultaneous trapping, with 9 in. suction trap and sticky trap of small Coleoptera on consecutive days*

Exp. 3*a*. Total numbers caught per hr. on 10 consecutive days

Hour ...	10–11	11–12	12–1	1–2	2–3	3–4	4–5	5–6
Suction	35	19	12	30	36	112	111	98
Sticky	5	1	2	12	4	10	6	11

Poor catching power might obscure events; Table 6 illustrates an obvious flight periodicity with small beetles which is shown by the suction but not by the sticky trap. The different density estimates for aphids with net and suction traps above 7 m.p.h. is another example, and is due to the net catching an occasional aphid while the suction trap catches none (p. 276). At low wind-speeds the situation is reversed; it is the suction trap which then catches at the higher rate. The small catch with the net is due to escape after capture or to dodging and not because the net samples less air than the suction trap; for actually it samples more even at 2 m.p.h. (Fig. 3). Escape is related to wind direction, for when the mouth of the net faces the sun the tendency for insects to escape is greater than when the wind turns the net in the opposite direction.

The accuracy of the density estimate

Aphididae, *Coleoptera*, *Chironomidae*. With these insects, all known to be affected by slight air movements, the suction trap gave higher values for density estimates than either of the other two traps in low wind-speeds (Figs. 1, 2, 6–8); the lower estimates with the sticky trap are probably due to more insects flying round the trap without being caught while with the net the low estimates are due mainly to escape after capture.

Preliminary experiments, which will be described in another paper, indicate that with aphids the suction trap is neither attractive nor repellent to a significant degree; indeed, the whiteness of the net and sticky trap should make these objects more attractive than the black suction trap (Broadbent, 1948). It is reasonable to suppose therefore, that since the suction trap does not suffer from weighting errors due to variation in the rate of air sampled, its density estimates, with aphids at least, are likely to be more accurate than those from the other two traps.

Chironomids are a somewhat special case since they may swarm above an object

such as a trap and be caught by the suction but not by the sticky trap; estimates of their density might thus have only a very local significance. On the other hand, if swarming is due to the warm air over an object the suction trap may not be biased in this way since there is a down-draught above it.

Insects other than aphids

With all other insects, except aphids, less than $\frac{1}{4}$ in. long, no statistically significant differences were to be observed between suction and sticky trap estimates of density over the wind-speed range in Exp. 2. Results from Exp. 3*b*, however, showed that the sticky trap gave higher values (cf. Figs. 4 and 8). These results were obtained in different years and in different places, and the discrepancy between the two experiments may have been due to differences in proportions of various insects in the two experiments. For the suction and sticky traps do not show constant differences between different kinds of insects. It is rather surprising to find, for example, that with the psocopteron the sticky trap gives the greater density estimates (Fig. 8). Now these insects are about as big as many aphids with similar flight speeds and apparently with about the same proportion of surface area to body weight. It seems unlikely that they would behave differently from aphids at the air-intake over the suction trap or in their impaction on to the sticky trap; it is more likely that the sticky trap is attractive to them. A similar argument might be applied to the Diptera and Hymenoptera which, as a combined group, also give higher density estimates on the sticky trap.

With larger insects (Fig. 5) the sticky trap again gives higher density estimates than the suction trap. Possibly the greater momentum of these larger insects gives them a greater degree of impaction, or since most of them were sarcophagids, muscids and syrphids, it may have been due to their habit of alighting on exposed surfaces.

It is clear from the above results that trap efficiency may vary for different kinds of insects and that at present few generalizations covering all kinds of insects are possible.

It is a pleasure to thank Mr R. Spacy who tended the trap at the Harpenden Sewage Works, Mr R. Marriott of Vent-Axia Ltd., Mr R. Taylor of Rothamsted Experimental Station for his assistance and Dr L. Lloyd for identifying chironomids.

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APPENDIX

Measurement of the percentage of wind passing through the net

The net, mounted on the cane hoop, was placed with its opening 12 ft. away from a large aerofoil fan working in a horizontal duct 18 in. in diameter. The speed of the wind made by this fan was measured with a Metrovic velometer at nine points across the two diameters at right angles within the cane hoop, with and without the net. This was repeated at different wind-speeds obtained by a pulley on the fan, which left the relative positions of the fan and net the same. The mean wind-speed at the net opening was expressed as a percentage of the mean wind-speed through the hoop without the net, for each wind strength. This is taken to correspond with the reduction in quantity of air sampled by the net; estimates of K for various wind-speeds are obtained from a smooth curve fitted to the data on p. 273.

SOIL SAMPLING FOR POTATO ROOT EELWORM CYSTS

A REPORT PRESENTED TO THE CONFERENCE OF ADVISORY ENTOMOLOGISTS

By F. J. ANSCOMBE
Statistical Laboratory, University of Cambridge

The National Agricultural Advisory Service (Advisory Entomologists) has adopted a uniform soil-sampling technique for potato root eelworm. In Part I the recommended technique is quoted in full. In Part II an account is given of some experimental sampling done by the Advisory Entomologists, on which the recommendations are partly based. In trying to estimate the average cyst content of the soil of a field, a statistical problem is encountered of deciding on an adequate sampling procedure. A similar problem was investigated in the wartime wireworm survey, but the rather different features of eelworm sampling call for fresh consideration of points of detail.

PART I. RECOMMENDED TECHNIQUE OF SAMPLING

In 1946 the Conference of Advisory Entomologists appointed a Subcommittee* to consider the determination of cyst populations of potato root eelworm (*Heterodera rostochiensis* Woll.) in soil. A uniform technique was needed, suitable for routine advisory work and for survey purposes. After discussing various techniques in use, and examining evidence of their reliability and convenience, the Subcommittee reported (May 1946) and made the following recommendations:

1. Field sampling

Instrument. 1½ in. soil auger or, alternatively, a half cylindrical sampler of 1 in. diameter (cheese sampler).

Depth of sampling. 8 in.

Number of samples. Fifty samples for fields up to 10 acres, larger fields to be subdivided into areas of 10 acres or less. In order to avoid subdivision where a field is only slightly over 10 acres, additional samples should be taken at the rate of ten for each additional 2 acres.

Procedure. The fifty samples should be taken more or less at random over the whole of the sampling area. In order to achieve this object, it is suggested that the sampling area should be split up into smaller areas of approximately equal size and a definite number of samples collected from each. For example, where the field is nearly square or rectangular, it would be convenient to divide it into four strips or lanes, and the sampler would pursue a zig-zag course up one lane, down the next, and so on, taking twelve to thirteen samples from each. In fields of irregular shape the sampler must use his discretion in splitting the sampling area, bearing in mind that the aim is to distribute the samples fairly.

Where any portion or portions of a field are known to have a history different from the remainder, these portions should be sampled separately.

* The members were: Dr H. W. Miles (Chairman), Mr F. G. W. Jones (Secretary), Mr F. J. Anscombe, Mr D. W. Fenwick, Mr L. R. Johnson and Dr I. Thomas.

2. Laboratory technique

Treatment of bulk samples. The fifty small samples taken in the field should be bulked together and the whole bulk-sample passed through a sieve of 2 mm. mesh, rejecting only the stones. The sieved sample should then be mixed by coning four times, after which a representative portion of rather less than 1000 g. should be extracted by the method of quartering. (Spread the soil into a circular mass some 2–3 in. deep. Divide into four quadrants and select one of these by tossing a coin. Retain this quadrant and the one diagonally opposite, but reject the other two. Mix the quadrants retained and repeat the whole process, continuing in this fashion until the quantity of soil remaining is approx. 1000 g.) This representative sample should now be placed in a shallow tray and air-dried at room temperature.

Flotation. Fifty g. of air-dried soil should be floated in a flask of 1000 c.c. capacity (round or conical). Decant twice on to a pair of sieves, the upper having 25 meshes/in. and the lower 80 meshes/in. Wash and transfer the contents of the lower sieve to a filter-paper or to bolting silk.

Cyst collection. The use of the collecting tray described by Fenwick (1940, pp. 170–1) is recommended. Where, however, the amount of debris in the float is small, cysts may be collected directly from the filter-paper. This operation is greatly facilitated if the filter is divided into sectors by means of a stamp (printer's line-block or rubber).

Cyst counting. The collected cysts are placed on a glass slide in rows in a small quantity of water or 20% glycerine. Count:

(1) The total number of spherical cysts. Lemon-shaped cysts must be neglected (i.e. those oval or subspherical cysts having, in addition to the neck, a second prominence bearing the vulva). On the question of the separation of spherical cysts into *H. rostochiensis* and *H. punctata*, Mr Fenwick said that omitting to do this was not likely to lead to an error greater than approximately 2% when potato fields were being sampled. The Subcommittee therefore recommends that such separation need not be attempted.

(2) The number of viable cysts (i.e. those spherical cysts containing one or more eggs or larvae), and divide them into two categories, (*a*) few eggs (1–20), and (*b*) many eggs (20+).

If the viable cyst count is less than 50 (1 viable cyst/g. of air-dried soil), then a second count should be made on a further 50 g. of soil. In deciding upon this procedure, the Subcommittee took into account the critical level (about 0·5 cysts/g. of air-dried soil) above which potato sickness is likely to occur, and the fact that counts of successive portions drawn from a thoroughly mixed bulk-sample should fit the Poisson distribution. From the latter it follows that the accuracy of a count is determined by the number of viable cysts counted. Thus with an average count of 50, the standard deviation is $\sqrt{50}$ = approximately ± 7, or $\pm 14\%$, and out of every 100 counts made at this level, about 75 would fall within $\pm 16\%$ and 95 within $\pm 28\%$ of the expected value 50. This degree of accuracy was considered to be sufficient for advisory purposes. In order to reach it at the critical level, two counts of 50 g. are necessary to give a total count of the order of 50 viable cysts.

For comparing two counts thus obtained, Mr Anscombe suggested the following quick test. Find the difference between the square roots of both counts. If this does not exceed 1·5, then the counts are in agreement. Occasional values greater than 1·5 may be neglected, but if they occur frequently it indicates that there is something wrong with the technique.

3. Investigation of field sampling

The Subcommittee experienced some difficulty in arriving at a decision as to the number of small samples to be taken per field and the area to which the number should be

limited. Figures bearing on this problem which had been submitted to Mr Anscombe referred mostly to small areas of 1 or 2 acres. The Subcommittee felt that further work on this point is desirable, and, in order to provide more information on this important aspect of the technique, it is suggested that during the winter of 1946/7 each Advisory Province should undertake the task of sampling one field of about 10 acres in the manner described above but keeping the small samples separate. After passing them independently through the 2 mm. sieve and air-drying, 50 g. of each sample should be floated and counted. The fields chosen for this purpose should have an eelworm population near the critical level and should not have grown potatoes in the previous season (1946).

4. Equivalence of different soils

Because equal weights of different soils occupy different volumes, the peats being notoriously light and bulky, it is suggested that when weighing out 50 g. of soil for flotation the volume of this mass of soil should also be measured by the following standard method: pour the 50 g. of air-dried soil into a standard 100 c.c. measuring cylinder and shake down by rapping the base of the cylinder smartly upon the bench three times. Read off the volume occupied.

5. Psychological errors in counting

In examining data submitted to him, Mr Anscombe found that the standard error of successive counts from the same bulk sample was, in some instances, only 80% of the expected value. Such a discrepancy might arise from a subconscious tendency to make counts agree. After making the first count, the operator might strive hard to collect the same number of cysts for the second and successive counts and, having obtained this number, might seek less diligently thereafter. Errors of this kind are to some extent avoided in the recommended technique by making only one or two larger counts and by separating the processes of collection and counting.

Note. Sieves of 2 mm. mesh aperture may be made from wire gauze or perforated zinc fastened to wooden frames. Sieves suitable for the flotation process are listed in dealers' catalogues under seed testing and metallurgical assay. They are cylindrical and fit into each other. Similar sieves may easily be constructed from wire gauze and sheet metal.

The above recommendations were adopted by the Conference.

Some fields were sampled experimentally in accordance with the suggestion in section 3. Owing to the severity of the weather, only six fields were sampled during the winter of 1946/7 (fields (i)–(vi) below). Further sampling was planned for the following winter, but was impeded by the reorganization that took place in the Advisory Services, in which a temporary dislocation of normal work was experienced; and a further six fields only have been sampled. From the observations on these twelve fields, it appears that the rate of sampling recommended above, namely 50 soil samples/10 acres, is perhaps unnecessarily high, and that if the rate of sampling were cut by a half the loss in accuracy would usually be slight. It is desirable not to have too high a rate of sampling, since the total weight of soil collected from a field may be considerable. Accordingly, at a meeting in March 1949, the Conference of Advisory Entomologists amended the above recommended technique of sampling by altering the paragraph on 'Number of samples' to:

Number of samples. Twenty-five samples for fields up to 10 acres, larger fields to be subdivided into areas of 10 acres or less. In order to avoid subdivision where a field is only slightly over 10 acres, additional samples should be taken at the rate of five for each additional 2 acres.

In view of the large amount of effort involved in the experimental sampling of fields, it was decided not to pursue the investigation further at present.

It must be emphasized that the above recommended technique of sampling is aimed at estimating eelworm populations in the neighbourhood of the critical level above which potato sickness is likely to occur. The critical level was estimated by Johnson & Thompson (1945) as 0·5 viable cysts/g. of dry soil, for a potato-growing area of Yorkshire, and this figure has been provisionally adopted as a standard for the rest of the country. The object of sampling for eelworm is usually to advise on whether a potato crop can safely be grown on the field in the next season. Fields of approximately the critical level of infestation were therefore chosen for the experimental sampling. But if the object of the sampling is to investigate very low infestations, as in testing the suitability of a field for growing eelworm-free stock seed, the problem is rather different. It is likely that more soil samples should be taken from the field, and it is certain that a considerably greater quantity of air-dried soil should be floated in the laboratory, if populations of the order of 0·01 viable cysts/g. of dry soil are to be investigated. The Conference has accordingly recommended the following increased rates of sampling in such work:

Number of samples. At least 50 samples for a field up to 5 acres; at least 100 samples for a field of 5–10 acres.

Flotation. At least 250 g. of air-dried soil to be floated.

PART II. STATISTICAL BASIS OF THE RECOMMENDATIONS

The standard error of the estimated mean infestation

The technique of sampling is: a certain number of borings (soil samples), say N borings, are taken from the field, at a fairly evenly spaced grid of points; the soil collected is dried and mixed in the laboratory, and one or more portions of 50 g. are weighed out, say n g. altogether; the cysts in each portion are counted by a flotation technique. Apart from possible errors of execution, such as inaccurate counting or identification of cysts, there are two sources of statistical error in the estimate of average cyst population per g. of dry soil that is obtained: (1) the density of infestation in the field may not be uniform, but vary from point to point; (2) the average density is estimated by flotation of only a small quantity of soil.

About the second of these errors a good deal is known. If the laboratory technique were perfect, in particular the mixing of the soil and counting of the cysts, counts from equal portions of soil from the same bulk-sample would be distributed in a Poisson distribution. This appears generally to be so, though, as noted above, some data came to hand showing too low a variance between duplicate counts. These were

counts of 20 g. portions of soil, made successively by the same person. It will be assumed below that such errors have been eliminated and the Poisson law holds.

Comparatively little is known of the uniformity of infestation in the field. Suppose a number of borings are taken at random from a particular area, and x g. of dry soil floated from each separately. If the average infestation is m cysts/g., the variance of the counts will be inflated above mx (the Poisson variance) by the unevenness of the infestation. We can define a measure k of the uniformity of infestation by setting the variance of the counts equal to

$$mx + \frac{(mx)^2}{k}. \tag{1}$$

For perfect uniformity $k = \infty$; the lower k is, the more uneven the infestation. We can interpret $k^{-\frac{1}{2}}$ as the coefficient of variation of the density of infestation from boring to boring; if a negative binomial distribution is fitted to the counts, k is the exponent of the distribution. If N borings are taken at random from an area having uniformity coefficient k, and after mixing the soil n g. are floated, the percentage standard error of the estimate of m so obtained is

$$100\sqrt{\left(\frac{1}{Nk} + \frac{1}{nm}\right)}. \tag{2}$$

This formula shows how the accuracy of estimating m depends on m itself, on the uniformity of infestation k, and on the amounts of field work (N) and laboratory work (n) that have been done. To decide what values to choose for N and n, in order to estimate a mean infestation m of any given size, we require to know what values of k are likely in practice.

The above relates to random sampling from the area. But in the recommended procedure the borings are taken, not at random, but in an evenly spaced pattern, forming a systematic sample.* The effect of this is to increase the accuracy of the estimated mean cyst population if there are any large-scale trends or patches in the density of infestation over the area. For example, if the northern third of the field carries a higher population of cysts than the rest, and if the borings are evenly spaced, very nearly a third of the borings will be in the high-density area, and hence that area will be fairly represented in the bulk sample, more so than it probably would have been if the borings had been taken strictly at random. For systematic sampling, therefore, the value of k to be inserted in formula (2) should represent the uniformity of infestation at *neighbouring* borings, made at the spacing actually used, and not the uniformity of infestation over the area sampled as a whole. For N evenly

* The wording of the Recommended Technique is not quite as clear as it might be on this point, for which I must take the blame. What is intended, and what is in fact done, is that the sampler should take borings as evenly as he can over the area, but without going to the trouble of measuring the positions of the borings exactly. To avoid any possible subjective bias in choosing the precise spot for a boring, the sampler may decide, after making each boring, how many paces he will take to the next, and then, having taken that number of paces, make the boring 1 ft. in front of his left toe (or some such rule). It is not likely, however, that subjective bias will be at all serious here, as it is in many other sorts of sampling.

spaced borings, from each of which x g. of dry soil are floated, we calculate the mean count $\bar{r}$, the variance s^2 of the counts, and a variance s'^2 calculated from differences between counts from neighbouring borings (i.e. the sum of squares of differences between counts from neighbouring borings, divided by twice the number of differences used). Then m is estimated as $\bar{r}/x$, the value of k appropriate to random sampling over the area is estimated as

$$k_1 = \bar{r}^2/(s^2 - \bar{r}), \tag{3}$$

while the value of k appropriate to systematic sampling with N borings is estimated as

$$k_2 = \bar{r}^2/(s'^2 - \bar{r}). \tag{4}$$

The latter estimate is not very exact, but good enough for present purposes. (The errors in systematic sampling have been considered in some detail by Yates, 1949.) If N is fairly large, a rough upper bound to the standard error of k_1 is

$$\left(1 + \frac{k_1}{\bar{r}}\right)\sqrt{\left(\frac{2k_1(k_1+1)}{N}\right)}, \tag{5}$$

this being in fact the approximate standard error of an estimate of k derived from N strictly random borings, when the counts follow a negative binomial distribution.

Experimental evidence concerning the value of k

The only counts of potato root eelworm cysts from single borings given in the literature appear to be counts of ten borings from each of two plots of 50–75 sq.yd. by Smith & Prentice (1929). One hundred c.c. of dry soil were floated from each boring. The mean counts were respectively 1·9 and 3·9 cysts/c.c. The values of k_1 were not significantly different, and gave a pooled value of 7, with standard error about 2·5. The authors quote a formula for the standard error of the mean count equivalent to formula (2) above. Other writers have given counts of bulk samples pooled from several borings, e.g. Hurst & Franklin (1938), who examined bulk-samples of eight borings from each of seventy-two plots of $\frac{1}{40}$ acre. But such data are not very helpful here, since neighbouring borings at such close spacing may be correlated, but the correlation is unknown. If a value of k_1 is calculated for Hurst & Franklin's bulk-samples, it will need to be divided by a factor lying somewhere between 1 and 8, to give a figure appropriate to single borings; the value 1 corresponds to perfect correlation between neighbouring borings, the value 8 to perfect independence; and this is a very wide margin of uncertainty. Jones (1945) has given counts of cysts, viable cysts, and eggs, of beet eelworm (*H. schachtii* Schm.) in two sets of forty-eight borings from a 2-acre plot. Considering viable cysts, the infestation was 2·0 viable cysts/g., and the pooled value of k_1 from both sets of borings was 9, with standard error about 1·5.

Thus, what little information has been available in the literature relates to small areas and rather high infestations. The programme of experimental sampling by the Advisory Entomologists was designed to give information directly relevant to

normal advisory work. The results of their investigation are summarized in Table 1.

Table 1

Field	Region	Area (acres)	No. of borings	Total viable cysts: Av. per g.	k_1	k_2
(i)	Yorks and Lancs	18	50	0·41	0·8	2
(ii)	West Midland	10	50	0·78	6	6
(iii)	East Midland	10	50	1·23	3	7
(iv)	Eastern	—	50	0·34	0·5	0·6
(v)	Eastern	—	50	0·56	2	2
(vi)	South-west	—	50	0·52	0·9	1·0
(vii)	Yorks and Lancs	6	50	0·40	1·4	4
(viii)	West Midland	8	50	1·17	1·2	1·6
(ix)	East Midland	12	60	0·45	17	60
(x)	South-west	7	50	0·37	2	2
(xi)	South-west	6	23	0·71	3	3
(xii)	East Midland	12	120	0·38	2	3

It will be noticed that there is a good deal of difference between these twelve fields in k_1 and k_2. One would like to see such observations on rather more fields before deciding how small a value of k should be guarded against in routine sampling. At present it seems reasonable to suggest that more often than not the appropriate value of k for even-spaced borings (i.e. k_2) will be at least 2, though lower values will be met sometimes. If the mean infestation m is 0·5 viable cysts/g., 100 g. of soil are floated, and $k=2$, the percentage standard error is

$$100\sqrt{\left(\frac{1}{2N}+\frac{1}{50}\right)}.$$

Field and laboratory errors will therefore be equal if $N=25$, i.e. twenty-five borings are taken per field. The standard error is then 20%. Increasing N to 50 only lowers the standard error to 17% unless more soil is floated. If, in fact, k is as low as 1, N would need to be doubled to achieve the same accuracy; with $N=25$ the standard error is 25%.

Thus with $N=25$ and $n=100$, the values now recommended, it seems we may expect the percentage standard error in estimating an infestation in the neighbourhood of 0·5 viable cysts/g. of dry soil to be about 20%, and usually there would be little gain in increasing N (without also increasing n). This accuracy is regarded as satisfactory.

Special investigations

(1) It has been argued that when fields are sampled at least a year after carrying potatoes, the part of the soil reached by cultivation should have been so much disturbed that the vertical distribution of the cysts is uniform. It will therefore make no appreciable difference whether borings are to 8 in. depth, as recommended, or to 4 in. only. To test this point, the first and second 4 in. of the borings were kept separate in two of the fields considered above, (viii) and (ix). In field (viii) there was

some evidence that the top samples were more heavily infested than the lower ones, roughly 25% more heavily. In field (ix) there was no discernible difference between the upper and lower samples. Pending further information, the question must remain open.

(2) In field (xii), of 12 acres, the sampling points were on an even grid at the high rate of 100/10 acres, and the borings were in triplicate: 'Normal', 'East' (taken 1 yd. to the east of Normal), and 'West' (taken 2 yd. to the west of Normal). There were thus 360 borings in all. The entries in the table above are averages for the three sets of 120. The only noticeable patchiness in infestation was a band of high density running across the middle of the field. There was no evidence of small-scale patchiness, and borings 1 or 2 yd. apart were no more alike, on the average, than borings many yards apart.

Sampling to measure very low infestations

The twelve fields sampled were chosen as having populations of the order of 0·5 viable cysts/g. Can we deduce anything from them about the standard errors to be expected when very low infestations such as 0·01 viable cysts/g. are to be measured?

Several sets of observations on insect populations that I have seen have suggested that for lower infestations a lower value of k is likely (Anscombe, 1949). In particular, an interesting parallel is provided by the wartime wireworm survey. Yates & Finney (1942) and Finney (1946) give graphs showing the relation between percentage standard error per core and mean infestation, derived from observations on more than 2000 fields.* If the ordinates of the graph (representing percentage standard error) are squared, the ordinates of the empirical error curve are $1/m + 1/k$, where m is the mean number of wireworms per core, while the ordinates of the Poisson-error curve shown also are $1/m$, so that $1/k$ can be read off as the vertical distance between the two curves. It is seen that k is not constant, but is about 3 for m between 2 and 4 wireworms/core (1 and 2 millions/acre), and lower for m lower, dropping to about 1·5 for $m = 0{\cdot}5$ wireworms/core. The variance per core can be graduated by the formula $m + 0{\cdot}45m^{1{\cdot}7}$, which is equivalent to setting $k = 2{\cdot}2m^{0{\cdot}3}$.

With eelworm cysts, therefore, we may expect that if m is very low k will be somewhat lower than the value 2 found to be typical when $m = 0{\cdot}5$ viable cysts/g. Having no direct information on the matter, we might make a not unreasonable guess and set $k = 0{\cdot}5$. We then have for the percentage standard error of estimating m, when $m = 0{\cdot}01$,

$$100\sqrt{\left(\frac{2}{N} + \frac{100}{n}\right)}.$$

Unless n is best part of 50 times N, nearly all the error is laboratory, not field, error. Such an unequal division of error between the two sources is probably economical of

* In the graph given by Yates & Finney (1942), and reproduced by Yates (1949), there is an error in the abscissa scale; the population figures are twice too large. Finney (1946) indicates the populations correctly, expressed as mean number of wireworms per core, instead of thousands per acre.

effort, in this case. For $N = 50$, $n = 250$, which are minimum values recommended for a 5-acre field, m is estimated with standard error 66%. The error can only be substantially reduced by a substantial increase in n.

It is interesting to consider what sort of guarantee of freedom from infestation is provided, when the soil floated is found to contain no cysts. This occurrence is rather unlikely (chance about 1 in 10) if the true population of the field is equal to 2·5 cysts per the weight of soil floated. For example, if 250 g. are floated, the occurrence is unlikely if the true population is 0·01 cysts/g. If, therefore, a considerable proportion of the fields sampled give no cysts in 250 g., we can feel fairly sure that most of these fields, at least, have populations substantially lower than 0·01 cysts/g. But if few fields, less than 10%, give no cysts in the 250 g. of soil floated, it may well be that all the fields have populations of over 0·01 cysts/g., the zero counts being merely accidental. If all the fields in a seed-growing area are sampled, it will be possible to construct a frequency table showing the proportion of fields giving 0, 1, 2, ... cysts in 250 g. From this we can get a fairly precise estimate of the average eelworm infestation in the area as a whole, and also see whether there appears to be any variation in infestation from field to field. If there is no such variation, the frequency table will follow a Poisson distribution,* while if some fields are in fact more heavily infested than others the frequency table should show a greater variance, and to some extent it will be possible to estimate the distribution of true population from field to field.

We may conclude, therefore, that the recommended sampling procedure is hardly satisfactory for inspection of individual seed-growing fields, but that it may prove useful in the survey of a seed district. The populations detectable by soil sampling are in any case high; a figure of 0·01 cysts/g. works out at something like ten million cysts/acre. To assert that the infestation of a field, or the average infestation of all the fields in a district, is less than 0·01 cysts/g., is by no means to say that there is no material infestation at all; and we cannot hope for a stronger guarantee than this from soil sampling without a very great increase of effort. The presence of a serious infestation can be demonstrated, but not its absence.

Another method of inspection for the presence of eelworm is by careful examination of the roots of a growing potato crop. The efficacy of this method is not under consideration here.

I am indebted to the Conference of Advisory Entomologists for permission to publish this paper, to individual advisers for supplying full information concerning the experimental counts, and to fellow-members of the Subcommittee for helpful criticism of the paper in draft.

* More exactly, a negative binomial distribution with high exponent. In view of the low mean count, the difference from a Poisson distribution will be negligible.

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NOTES ON THE FEEDING HABITS OF HOUSE-RATS IN RANGOON, BURMA

By J. L. HARRISON* and H. C. WOODVILLE†
Formerly of No. 1 Pest Destruction Advisory Unit, S.E.A.C.

(With 4 Text-figures)

During a poison campaign against house-rats, particularly of the species *Bandicota bengalensis*, *Rattus rattus* nr. *khyensis* and *R. exulans concolor*, a number of incomplete experiments were carried out on feeding habits and the take of poison.

The rats showed a reaction to new objects similar to that of *R. norvegicus* in England.

A marked preference was shown for boiled rice over any other available foodstuff. The amount eaten was roughly proportional to the surface area of the rat. Barium carbonate in boiled rice was an acceptable poison bait, and the optimum concentration of poison was in the range 10–20%.

Extensive rat poisoning was tried in Rangoon in 1945 for the control of a small outbreak of bubonic plague. The methods used and the results have been reported by us (Harrison & Woodville, 1948). Although the technique of prebaiting was well known at that time it had not been tried against rats of the species occurring in Rangoon, and certainly very little was known of the feeding habits of these rats. During the course of the campaign numerous feeding tests were performed, but unfortunately it was not possible to continue and complete the investigations.

Species present

Rats of the following species were present in the town:

Bandicota bengalensis (Grey & Hardwicke), the lesser bandicoot or indian mole rat. This large ground-living rat, which made extensive burrows, formed about 30% of all rats seen.

Rattus norvegicus (Berkenhout), the cosmopolitan brown rat. This rat was confined to the docks and town area where it formed about 10% of all rats seen.

R. rattus form near *khyensis* Hinton, a form with a bright brown dorsum and a clear white venter. It seemed to live in trees and better-class houses (with an upper story), and formed about 10% of all rats seen.

R. exulans concolor (Blythe), the little Burmese house-rat. This rat was abundant in houses of all kinds and comprised 40–50% of all rats seen.

Mus musculus Linné, the house-mouse, formed only about 5% of the rodent population.

* Now at Institute for Medical Research, Malaya.
† Now of National Agricultural Advisory Service, Staplake Mount, Starcross, Exeter.

The house-shrew, *Suncus caeruleus* (Kerr) (Insectivora, Soricidae), was also abundant, forming about 10% of all animals trapped.

The important species appeared to be *Bandicota bengalensis*, *Rattus rattus* and *R. exulans*, and control measures (and associated tests) were directed principally against these. Where 'rat' is used in this paper without qualification it is meant to apply to these three species only. *R. norvegicus* did not occur in the plague area (Harrison, 1946). It will be observed that the form of *R. rattus* present was of the white-bellied type, usually associated with forest and fields. Rats of the dark-bellied type (*R. rattus rattus*, *R. r. alexandrinus*, *R. r. rufescens*) were not seen.

Feeding

Food preference. Preliminary tests were made on wild colonies to see which of the available foodstuffs were preferred. Foods which were available in sufficient quantity were: polished rice; a wheat wholemeal called atta; flour (wheatmeal of about 75% extraction); and dhall (dried pulse) of two kinds—chenna (*Cicer arietum*) = chickpea, and moong (*Phaseolus* spp.) = green gram.

The rat colonies were offered a choice of these, and the weight of each taken was noted. Baits were laid in a tray with five compartments and to avoid the effect of place preference, the positions relative to one another were varied, with the aid of a table of random numbers. The differences of amounts of the different foods taken over a period of 10 days were tested by Student's *t* test and if the chance of such difference were less than 5% the foods were given different ranks of preference. The ranks obtained are shown in Table 1.

Table 1. *Showing the preference for foodstuffs of* Rattus rattus, R. exulans, Bandicota bengalensis *and* Mus musculus

(Each foodstuff is marked in the order in which it was preferred (1 means first preference). *X* means that none of that particular food was taken when offered, a dash means that none was offered.)

Test	Rat species	Rice	Flour	Atta	Chenna	Moong
1	*R. rattus* with some *R. exulans* (wild)	1	3	3	5	1
2	*R. rattus* with some *R. exulans* (wild)	1	3	2	5	4
3	*B. bengalensis* in an enclosure	1	3	2	*X*	4
4	*B. bengalensis* in an enclosure	—	1	2	3	3
5	*B. bengalensis* in an enclosure	—	1	2	3	*X*
6	*R. exulans* (cage)	1	—	*X*	—	*X*
7	*M. musculus* (wild)	1	—	2	3	—

It will be seen that there was a decided preference for rice. It was found, moreover, that rice would attract rats away from a point at which they were accustomed to feed. One of the most conspicuous examples of this was obtained in the Zoological Gardens where a wild colony of *R. rattus* and *R. exulans* was feeding on atta. A sudden drop in the daily take was caused when a Macaw was moved into a nearby cage and fed on paddy (unhusked rice). When rice was provided at the baiting point

the daily take recovered its former level. Other examples are among results deposited in the University of London Library (Harrison, 1947).

Raw and boiled rice. Because poison will not stick to dry grains the rice was boiled. It was found by many tests that boiled rice was in fact preferred to the raw grain. A single test suggested that *Mus musculus* did not have similar preferences.

The daily take of boiled rice was found to be greatly in excess of other foodstuffs. By feeding caged rats successively on boiled and raw rice it was found that the dry weight taken of each was approximately the same. Table 2 gives the result of twelve such tests and a *t* test does not reveal any consistent difference.

TABLE 2. *Relative take of raw and boiled rice by* Rattus rattus, R. exulans, Bandicota bengalensis *and* Mus musculus

			Mean take during successive periods			
			Gross weight taken		Dry weight taken	
Test	Rat species	No. of rats	Boiled	Raw	Boiled	Raw
1	*B. bengalensis*	1	34·0	9·6	8·1	8·1
2	*B. bengalensis*	1	18·0	7·0	4·3	5·9
3	*R. rattus*	2	32·0	9·6	7·7	8·1
4	*R. rattus*	1	17·5	6·0	4·2	5·1
5	*R. exulans*	1	16·4	3·7	3·9	3·1
6	*R. exulans*	1	42·0	9·0	10·1	7·6
7	*R. exulans*	1	22·5	9·0	5·4	7·6
8	*R. exulans*	1	14·0	4·5	3·4	3·8
9	*R. exulans*	3	33·0	9·0	7·9	7·6
10	*R. exulans*	3	30·0	7·5	7·2	6·3
11	*R. exulans*	1	11·0	3·0	2·6	2·5
12	*M. musculus*	4	37·6	8·4	9·0	7·1

TABLE 3. *Total uptake of water by* Rattus rattus, R. exulans *and* Bandicota bengalensis

				Mean take of			Total water	
Test	Rat species	Wt. of rat	Foodstuff	Food (g.)	Water (c.c. = g.)	Water in food	In g.	As percentage dry wt. of food
1	*B. bengalensis*	120	Atta	8·3	10·3*	1·6	11·9	178*
2	*B. bengalensis*	37	Raw rice	6·2	3·3	1·0	4·3	83
			Atta	8·5	4·7	1·6	6·3	91
3	*R. exulans*	*c.* 40	Atta	4·9	4·3	0·9	5·2	127
			Raw rice	3·5	2·6	0·6	3·2	110
4	*R. exulans*	40	Raw rice	3·6	1·9	0·6	2·5	83
			Boiled rice	13·0	0·5	9·9	10·4	335
5	*R. rattus* (2)	(100) (28)	Raw rice	9·3	4·9	1·5	6·4	82
			Boiled rice	31·9	0·0	24·3	24·3	333

* Possibility of a leak giving these high figures.

It is noteworthy that boiled rice was available to all wild town rats. The staple diet of the human population was rice, and no one seemed satisfied with his meal unless some of the rice was left over. This was usually dropped outside the house to be eaten by dogs, crows, or rats.

Water requirements. It was found that rats fed on a diet of boiled rice seemed to require no drinking water. A few measurements of water uptake are summarized in Table 3. These tests were performed in July and August when the temperature varied only from 27 to 30° C. and the R.H. was 75–90%. In these circumstances boiled rice appeared to provide more than enough water.

Food and body weight. Boiled rice was made the standard bait, and in the course of other tests the relation between average weight of rice eaten and the weight of the rat was calculated. It appeared to be of the exponential form

$$\text{take} = (\text{body wt.})^x,$$

where x was of the order of 2/3. That is, if weight is taken as a measure of volume, the uptake of boiled rice was roughly proportional to the surface area of the body.

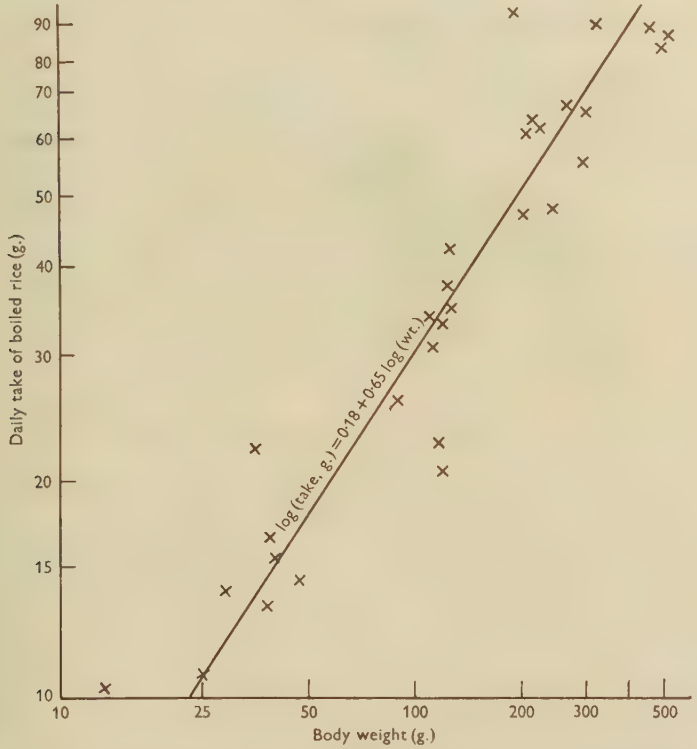

Fig. 1. Relation between body weight and take of foodstuff. Average daily take of boiled rice plotted against body weight, log.-log. scale. All species.

Fig. 1 shows the average daily take of boiled rice, for rats of all species, plotted against body weight on logarithmic scales. The calculated straight line of best fit is drawn in; it is the line

$$\log (\text{take in g.}) = 0{\cdot}18 + 0{\cdot}65 \log (\text{body wt. in g.}).$$

The corresponding line for dry weight of rice eaten is

$$\log (\text{dry wt. in g.}) = \bar{1}{\cdot}56 + 0{\cdot}65 \log (\text{body wt. in g.}),$$

and one of us (J. L. H.) has found that the average takes of raw rice by specimens of *Rattus rattus diardi*, *R. r. argentiventer*, *R. r. jalorensis* and *R. exulans*, in Malaya are also fitted by this line.

Other foodstuffs. The relative uptakes of rice and other foods were compared by feeding rats on one foodstuff at a time for periods of from 5 to 10 days and comparing the average daily take after the first 3 days on each food. The feeding for the first few days is likely to be affected by preference. Results in terms of dry weight of food, taking rice as 100, are given in Tables 4 and 5. The 'average' is the arithmetic mean of the percentages, excluding data for *Mus musculus* which appears to have preferences rather different from those of the rats.

TABLE 4. *Total takes of different foodstuffs of rats in cages or enclosures; no other food available*

(Mean takes of food during successive periods, dry weights expressed as percentages of raw rice taken.)

Rat species	No. of tests	Raw rice	Boiled rice	Atta	Moong	Chenna
B. bengalensis in enclosures*	6	100	—	140–148, 196	75, 155	—
B. bengalensis juv. in cage*	2	100	89–100	74, 118	37, 58	—
R. rattus	1	100	82	—	—	82
R. exulans	4	100	126, 133	116–136	97	—
M. musculus	3	100	127	125–201	110	—
'Average' for rats		100	106	133	84	82

* Adult *B. bengalensis* could not be kept in cages.

TABLE 5. *Total take of different foodstuffs of rats in wild colonies; other food presumably available*

(Mean takes of food during successive periods, dry weight as percentage of raw rice taken.)

Species in colony	Raw rice	Boiled rice	Atta	Moong	Chenna
B. bengalensis	100	—	—	96	59, 64
R. rattus	100	—	61, 109	—	—
R. rattus and *R. exulans*	100	—	128–151	35, 43	—
B. bengalensis and *R. exulans*	100	—	—	37	146
M. musculus	100	76	70, 145	—	46
'Average' for rats	100	—	116	53	90

The results appear to show that on a dry-weight basis, rats require more atta than

rice but less dhall, especially when other food is available (as in wild colonies). A possible explanation of these differences is found in the food values. Table 6 compares the averages taken with the weights needed to provide the same calorific value and the same protein value. Differences of calorific value provide a plausible explanation of the slightly greater uptake of atta, whereas a similar uptake of dhall would provide an excessive amount of protein.

TABLE 6. *Total take compared with food value: relative dry weights of foodstuffs required to give equal calorie value and equal weight of protein (rice as* 100*) compared with the 'average' takes from Tables* 4 *and* 5

	Rice	Atta	Moong	Chenna
Average take in cages (T. 4)	100	133	84	80
Average take, wild (T. 5)	100	116	53	90
Wt. needed for:				
Equal calorie value*	100	111	113	103
Equal wt. of protein*	100	69	34	37

* Figures from Platt (1945).

Mixed feeding. When a sufficient supply of several foodstuffs was offered, a single rat would sometimes eat only the preferred food as in Table 1, test 6. Usually it would divide its take, eating more of the preferred food, but some of the others. Table 7 gives some examples of this mixed take of rice and atta. These few examples suggest that a larger gross weight of the preferred foodstuff was eaten, irrespective of the water content.

TABLE 7. *Shared take, rice and atta presented together: mean take in g. of each food when both were taken*

Test	Rat species	Raw rice	Boiled rice	Dry wt. of rice	Atta
1	*B. bengalensis*	26·0	—	22·0	4·3
2	*R. rattus*	—	16·7	3·3	2·5
3	*R. exulans*	—	12·0	2·9	6·8
4	*R. exulans*	—	4·7	1·1	2·0

Reaction to new baits. When a bait was presented in a new place it was found, as with *Rattus norvegicus* in England (Anonymous, 1939–46), that the amount taken increased daily over a period of some 3–5 days, and then oscillated about a fixed level. The level might change subsequently, presumably with changes in the constitution of the rat colony, but a level of sorts was usually discernible.

This slow build-up of take appeared, as in *R. norvegicus*, to be caused by three factors: (*a*) a delay in the rats finding the baiting point; (*b*) a tendency to feed at the same place each night; and (*c*) avoidance of new objects or foodstuffs. The operation of all three factors was discernible in the three species tested (Harrison, 1947). Fig. 2 shows an example of the reduced take consequent upon a slight change in the nature of the baiting point.

Poisoning

At the time the only poison available in any quantity was barium carbonate of uncertain purity and toxicity. Luckily boiled rice is a convenient base, because a large volume is taken, the poison sticks to the grain, and the whole grain, including the outer surface is eaten. In view of the preference for boiled rice shown by the rats, and the large amounts available to them (page 299 above), no other bait was likely to be successful.

To see if barium carbonate on boiled rice was acceptable, and to decide on the best proportion of poison in bait, a number of test poisonings were made on rats in cages and in small enclosures. The rats were fed on boiled rice for 5–10 days and

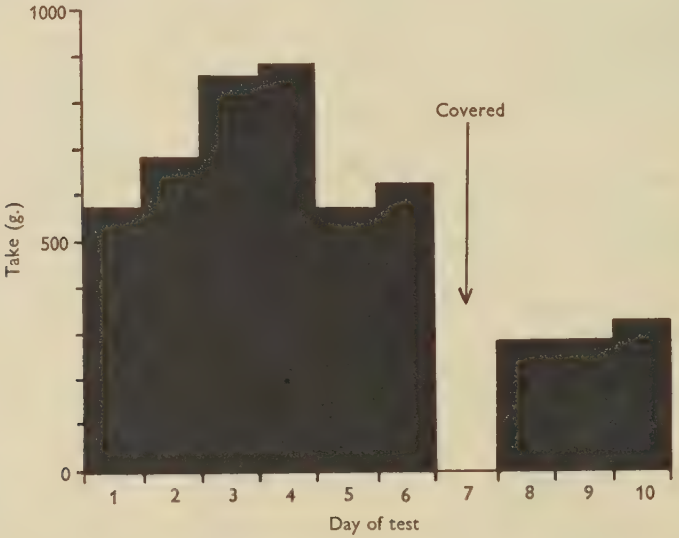

Fig. 2. Avoidance of a new object. Daily take of rice from an open tray (wild mixed colony). On and after day 7 the tray was covered with an inverted box with holes cut for entrance.

then, on the following night offered the poison mixture to be tested. At first, following the Royal Army Service Corps' Training Manual, instructions for use of barium carbonate in breadmash, 20% of poison was used, later, other proportions were tried.

Thirty-four poison tests were made on single or grouped rats which, as far as was known, had had no previous experience of poison. All five species were tested, and individuals of each species accepted poison bait and died. No systematic attempt was made to determine the toxicity of the poison, but it was noted that of thirty-three rats consuming more than 1 g. of poison/kg. body weight only one survived, whereas of six consuming less than 1 g./kg. four survived.

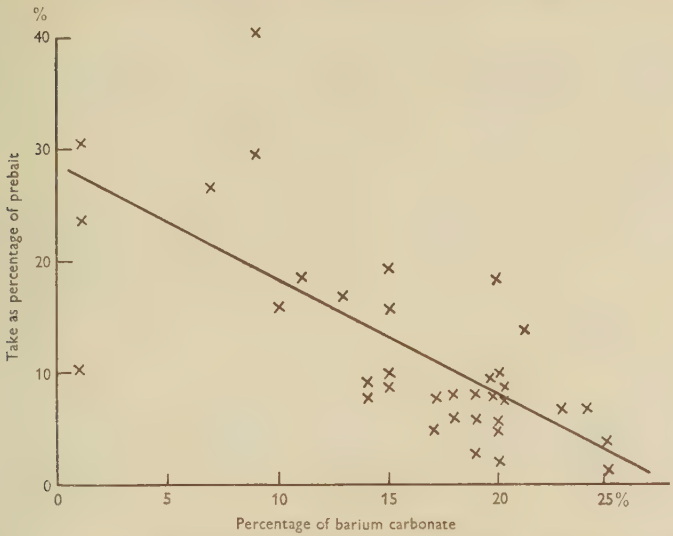

Fig. 3. Relation between bait take and concentration of poison. Each cross represents the amount of poison bait of corresponding strength taken by a caged rat with no experience of poison. Take is expressed as a percentage of the average daily take of unpoisoned food.

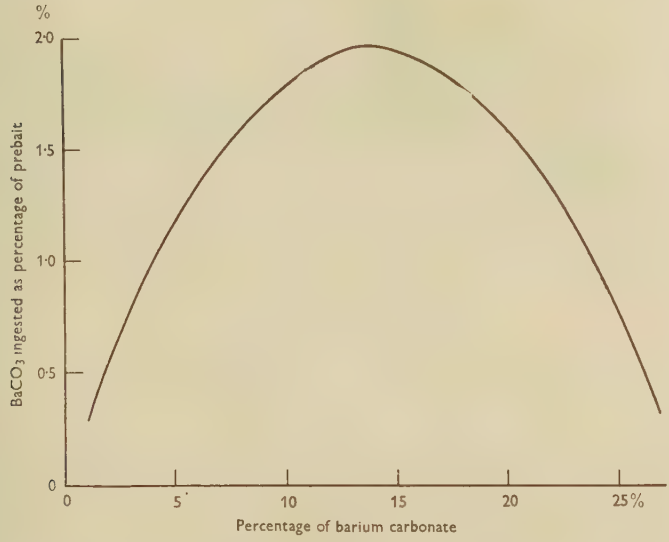

Fig. 4. Relation between poison ingested and concentration. The straight line of best fit in Fig. 3 replotted to show the amount of barium carbonate in the poison bait actually taken, also expressed as a percentage of the average daily take of unpoisoned food.

It became evident that the amount of poison-bait taken varied with the proportion of poison in the bait. Fig. 3 shows the amount of poison bait taken, expressed as a percentage of the average take of unpoisoned rice, plotted against concentration of poison. Although the points are scattered, the mean takes at various concentrations suggest that a simple linear relation is the best first approximation, and the calculated line of best fit is drawn in.

If the relationship of take to concentration is indeed of this form, i.e. a straight line or an approximation to one, then there will be an optimum concentration at which the amount of poison ingested is at a maximum. To illustrate this the straight line of best fit in Fig. 3 is replotted in Fig. 4 on a scale of actual poison ingested (not poison bait) against concentration. The maximum at a concentration in the range 10–20% is striking.

The mixture adopted for full-scale poisoning was one part of barium carbonate to five parts of boiled rice. In theory this should have given a concentration of 16·7%, but since in practice a proportion of the poison sticks to the sides of the mixing vessels (in our case, petrol tins), the resulting concentration was a little lower.

Effect of poison on survivors. Rats which survived a dose of barium carbonate varied in their reaction to further baits of the same poison. Some accepted a subsequent lethal dose, some refused to eat poisoned rice, but most accepted the bait but showed a marked resistance to the effect of the poison. Experience of barium carbonate in rice seemed to have no effect on the subsequent take of rice either plain or poisoned with arsenic.

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PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

Ordinary Meeting of the Association held on Friday, 4 November 1949, in the Imperial College of Science and Technology, London; the President, Mr G. Fox Wilson, in the Chair.

The following papers were read and discussed:

1. Mosquito eradication campaigns in Cyprus and Sardinia. By Dr J. R. Busvine.
2. D.D.T.-resistant house-flies. By Miss C. M. Harrison.

Ordinary Meeting of the Association held on Friday, 2 December 1949, in the Imperial College of Science and Technology, London; the President, Mr G. Fox Wilson, in the Chair.

Clover breeding, diseases and pests

The following papers were read and discussed:

1. The present position in the breeding of herbage legumes. By Mr Watkin Williams.
2. Clover seed weevils. By Mr J. Morgan Jones.
3. Clover sickness. By Dr W. A. R. Dillon Weston.
4. Stem eelworm and clover. By Dr T. Goodey.
5. Non-parasitic clover sickness. By Dr H. H. Mann.
6. Recent investigations on clover rot. By Mrs M. Justham and Mr L. Ogilvie.

MOSQUITO ERADICATION CAMPAIGNS IN CYPRUS AND SARDINIA

By James R. Busvine, *London School of Hygiene and Tropical Medicine*

The aims and principles of mosquito eradication programmes were described with a brief historical background. Details of the two latest campaigns were given with short descriptions of the islands concerned (see *Nature, Lond.*, **161**, 7 February 1948).

Two 16 mm. silent films were shown to illustrate various phases of the work, e.g. (1) preliminary entomological survey, (2) anti-adult house spraying, (3) the main, anti-larval campaign, (4) checking the progress of eradication. The Sardinian film was in black and white; the Cyprus film in Kodachrome.

D.D.T.-RESISTANT HOUSE-FLIES

By C. Mary Harrison, *London School of Hygiene and Tropical Medicine**

The resistance of insects to insecticides is by no means a new phenomenon. As long ago as 1914 there were reports of scale insects resistant to lime sulphur spray (Melander, 1914). Again in 1916, in California, the red scale *Aonidiella aurantii* was found to be resistant to hydrogen cyanide (Quayle, 1938). Normally one fumigation was effective for 2 years, but in some areas it was necessary to carry out fumigation twice annually and at an increased dosage. Hough (1928) found there was a difference in the ability of larvae of the codling moth *Carpocapsa pomonella* to enter apples sprayed with lead arsenate. He compared strains from Colorado and Virginia and found larvae of the Colorado strain could penetrate fruit more readily.

The possibility of insects becoming resistant to insecticides was thus well established long before there were reports of D.D.T.-resistant house-flies. The first published record of D.D.T. resistance in the house-fly relates to 1946. Wiesmann (1947) compared strains of flies from Basle, Switzerland and Arnäs, Sweden, and found the Swedish strain to be highly D.D.T.-resistant. The Swedish flies, when exposed to D.D.T. in such a way that only the tarsi came into contact with the insecticide, could withstand a concentration of D.D.T. 100 to 200 times greater than the Basle strain. Since this record, there have been numerous reports, both verbal and published, of resistant flies in widely separated parts of the temperate zone of the Northern hemisphere. The failure of D.D.T. to control house-flies in Italy was noted by members of the Istituto Superiore di Sanità, Rome (Sacca, 1947). Hadjinicolau (1948) states that D.D.T. gave good control of flies in the first year of spraying in certain districts of Greece but that in the second year of spraying complaints of inadequate fly control were numerous. He does not state that the flies had become D.D.T.-resistant but this seems highly probable. More recently, reports of D.D.T. resistance have come from Denmark (Keiding & Van Deurs, 1949). The use of D.D.T. against flies on farms in Denmark was started on a small scale in 1944 but by 1946 this insecticide was used extensively. There were some reports of failure of D.D.T. to control flies in 1946, and by 1947 many such reports. America, too, has its D.D.T.-resistant house-flies. King & Gahan (1949) tested samples of flies from five states of the Union and found all the samples to be more resistant to D.D.T. residues than flies of two laboratory strains which had never come in contact with D.D.T. Also from America are records of artificially produced D.D.T.-resistant strains. Lindquist & Wilson (1948) produced a resistant strain by exposing flies to an atomized mist of D.D.T. in kerosene and breeding from the surviving flies. A repetition of the process of selection through fourteen generations produced a markedly D.D.T.-resistant strain of flies.

Considering the problem of D.D.T. resistance in the house-fly from the practical standpoint it would be of value to know:

(1) whether the resistance to D.D.T. would decline if spraying with D.D.T. was discontinued;

(2) whether there are insecticides which will kill D.D.T.-resistant flies;

(3) if other insecticides are used, whether there is a possibility of flies becoming resistant to them also, and if so, how long it would take for such strains to develop.

(1) A comparison of two strains of flies, one D.D.T.-resistant and the other non-resistant to D.D.T., obtained from the Istituto Superiore di Sanità, Rome, has been made at the London School of Hygiene and Tropical Medicine. Samples of flies of these two strains were exposed to D.D.T. residues on Essex board for 1 hr., and a mortality count made 24 hr. after the exposure. The concentration of D.D.T. required to give a 50 % kill in the non-resistant strain was 40 mg./sq.ft.; and in the resistant strain 158 mg./sq.ft. Flies of the resistant strain could therefore withstand a concentration of D.D.T. approximately four times greater than the non-resistant strain.

* Working on a grant from the Medical Research Council.

These two Italian strains of flies were cultured in the laboratory without coming into contact with D.D.T. for 6 months. A repetition of the test comparing resistance of the two strains showed that there had been a definite decline in the resistance of the resistant strain, which was now only 1½ times as resistant as the non-resistant strain. After a further 6 months, i.e. after culturing for twenty-six generations in the laboratory, the resistant strain was only 1·2 times as resistant as the non-resistant strain.

There is similar evidence from Denmark (Keiding & Van Deurs, 1949) of a decline in resistance of flies after culturing over several generations without coming into contact with D.D.T., and from America (Barber & Schmitt, 1949).

(2) Observations on the effectiveness of insecticides other than D.D.T. on D.D.T.-resistant flies may be divided into two categories: those referring to flies obtained from districts where D.D.T. spraying has been widespread, and those referring to laboratory-developed D.D.T.-resistant flies. There is evidence that the resistance of 'wild type' D.D.T.-resistant flies is of a more specific nature than that of strains produced in the laboratory by selection. An analysis of results obtained in America (Barber & Schmitt, 1948; King & Gahan, 1949), Denmark (Keiding & Van Deurs, 1949) and of field observations from Italy (Mosna, 1949), shows that 'wild type' D.D.T.-resistant flies are not resistant to benzene hexachloride, chlordane or toxaphene, but where tests were carried out with analogues of D.D.T. the 'wild type' D.D.T.-resistant flies were resistant to these also. In contrast to this, there is a record of an artificially produced D.D.T.-resistant strain which proved to be resistant to a number of different insecticides (Wilson & Gahan, 1948; King, 1948). This selected strain of house-flies was resistant to methoxy D.D.T., Thanite, benzene hexachloride, pyrethrum, rotenone, chlordane and toxaphene, although the degree of resistance to these other insecticides was not as great as it was to D.D.T. It does not seem unlikely that during the selection for D.D.T. resistance a selection of flies which were generally 'stronger' than normal may have taken place.

Tests carried out at the London School of Hygiene and Tropical Medicine with the γ isomer of benzene hexachloride, on the two Italian strains of flies, gave rather variable results, but neither strain was consistently more resistant to this insecticide than the other. Preliminary experiments involving the injection of chlordane into flies of these two strains show that D.D.T.-resistant flies are not resistant to α benzene hexachloride. The D.D.T.-resistant flies, however, are more resistant to pyrethrins than flies of the non-resistant strain.

(3) The possibility of raising strains of house-flies resistant to D.D.T. by means of selection is well established. An appreciable resistance can be obtained by selection over a very few generations. By exposing flies of our non-resistant Italian strain to filter-papers impregnated with D.D.T. in each of three generations and breeding from the surviving flies there was a definite increase in resistance to D.D.T. in each of the three generations. A similar process of selection in the already resistant Italian strain gave a considerable increase in resistance. Before selection, exposure to filter-paper impregnated with 1·0 mg./sq.cm. of D.D.T. for 1 hr. gave an 80 % kill, but after selection for eight generations exposure to this dosage gave no kill. Further, experiments now in progress show that it is possible to raise a strain of flies resistant to pyrethrins from the non-resistant Italian strain. The time required to give a 50 % knockdown in the selected strain expressed as a percentage of the time required to give a 50 % knockdown in the normal strain in each of four generations is as follows:

Generation 1	109	Generation 3	147
Generation 2	118	Generation 4	250

This pyrethrin-resistant strain is also more resistant to D.D.T. than the non-resistant strain.

Attempts to produce a strain of flies resistant to benzene hexachloride from the non-resistant Italian strain have so far failed. Selection was carried out over five generations, but in the sixth generation the resistance was found to be no greater than when selection was started.

There is, as yet, no satisfactory explanation of the physiological mechanism of D.D.T. resistance in the house-fly. Wiesmann (1947) found morphological differences between

resistant and non-resistant flies. The D.D.T.-resistant flies examined had a thicker tarsal sole, stronger tarsal hairs, greater pigmentation of the tarsi, and a smaller absorbent surface of the tarsi. Wiesmann then suggested that differences in resistance to D.D.T. were explicable on the grounds of these morphological peculiarities. If, however, flies of the resistant strain are more resistant to injected D.D.T. than non-resistant flies, any correlation between thickness of the cuticle and D.D.T. resistance would be fortuitous and not causal. A greater resistance to injected D.D.T. by D.D.T.-resistant flies was first demonstrated by Bettini (1948). Injection experiments on our two Italian strains corroborate Bettini's evidence that D.D.T.-resistant flies are more resistant to D.D.T. on injection than normal flies. It seems that D.D.T. resistance is due to something more intrinsic than a greater thickness of the cuticle and area of absorption of the tarsi. There is obviously considerable scope for further research in this field.

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In the subsequent discussion Messrs F. G. S. Whitfield, A. H. Baker and G. F. M. Whitley gave the following account of observations they had made on D.D.T.-resistant house-flies.

During the past summer we were asked to investigate a number of cases where continuous-flow aerosols of D.D.T. were failing to kill the common house-fly.

Factors which might have caused these failures were:

(1) *The high ambient temperature.* The failures occurred during the height of the summer, and as we have both field and experimental evidence that high temperatures have an adverse effect upon the operation of D.D.T. against certain insects, this factor could not be ignored.

(2) *Deterioration of the insecticide.* The quality of the D.D.T. concerned was identical with that used successfully in large numbers of other installations.

(3) *Insufficient dosage.* This was taken into account, and while it was undoubtedly a factor to be considered in some installations, was not operative in the cases described.

(4) *Very favourable breeding conditions.* An obvious factor tending to operate against the 100 % control of large fly populations.

Having taken these factors into account there remained one or two cases which merited further investigation and two of these proved to be instances of resistant flies. The essential data are as follows:

The localities in question were at Stratford-on-Avon and at a farm near Winchester.

In the Stratford-on-Avon area specimens were collected from three premises, two being kitchens of hotels and one the public rooms of a cafe, all being within a half-mile radius. In the Winchester farm the flies were taken from the calf-house, the cowhouse and from one or two outside walls.

The flies from both areas were bred in the laboratory and after oviposition some of the adults were used for preliminary tests.

A generator was set up to produce 6 mg./1000 cu.ft./hr. in one of our experimental chambers and after running for half an hour a dozen adults of a standard laboratory strain were liberated in the chamber. These succumbed within 30 min. and were then removed and immediately replaced by a dozen of the original Stratford strain which after 18 hr. were still active and flying though one or two were showing slight tremors. Throughout this period the aerosol was in continuous production, so that a steady build-up was taking place and the Stratford strain were subjected to appreciably higher dosage than the laboratory strain. The conditions of the test were air temperature 21° C., R.H. 55 %.

Only one further test with this strain has since been possible owing to its slow breeding rate. Forty-eight flies of the F_2 generation were divided equally between two identical 1 m. chambers in a room thermostatically controlled at 21° C. One batch received a dose of 4 mg. of laboratory, twice recrystalized *p. p'*-D.D.T./cu.m., the other half this amount. The insecticide was used as a 1 % solution in acetone and volatilized from a nickel capsule on an electric strip heater. The amount of acetone present was only an insignificant fraction of that required to show any toxic response. The resultant knockdown in 60 min. without recovery was 45·8 and 25 % respectively. The responses of the normal laboratory strain to half the above concentrations are 99·7 and 95 % respectively.

The Winchester strain of flies has been subjected to tests in batches of 24 in the 1 cu.m. chambers at the 3rd, 4th and 5th generations with the following results:

Dose	Knockdown	
4 mg./cu.m.	18 20 17	L.D.$_{50}$ 2·9 mg./cu.m. limits (2·04–4·25)
2 mg./cu.m.	5 4 12	

A statistical assessment of the results is given in Table 1.

Table 1. *Assessment of results of insecticidal tests with three different strains of house-flies*

Strain of house-fly	50 L.D./1 hr. (mg./cu.m.)	Slope
Laboratory	0·436 (0·13–0·96)	3·632
Stratford	3·03 (1·88–4·9)	1·894
Winchester	3·12 (2·03–3·82)	5·00

There seems little doubt, from the above evidence, that strains of D.D.T.-resistant *Musca domestica* are now occurring in this country. The two strains investigated show that about seven times the dose of D.D.T. applied as a continuous-flow aerosol without carriers or solvents is required for the L.D.$_{50}$ as compared with our stock strain. Another point not shown in Table 1, in support of this conclusion, is that no knockdown occurred with the resistant strains for about 45 min., whereas the laboratory strain showed casualties in approximately 20 min. with a 50 % knockdown at 30 min. from 1 mg./cu.m.

THE PRESENT POSITION IN THE BREEDING OF HERBAGE LEGUMES

By Watkin Williams, *Welsh Plant Breeding Station, Aberystwyth*

The progress due to plant breeding in the last three decades or so has given to agriculture most of the strains of the traditional species of herbage legumes that it requires for efficient agricultural production. The most striking of these developments has been in the white clover species, where the pedigree big-leaved strains have brought about a radical change both in the production and utilization of the crop. A similar, though not nearly so spectacular development, has been the selection of the extra late-flowering type of red clover from local races in this country, whose special features remained undiscovered except to a small number of farmers until plant breeding was commenced on the species.

The experience gained in clover improvement during the last 30 years or so shows fairly clearly two important points. First of all, improvement has been effected only from ecotype populations which were already in existence, and which had reached a remarkable degree of natural stability both in type and performance. Secondly, when these natural ecotypes have subsequently been carefully multiplied, the ecotype has in most cases approached the pedigree material in the main important agronomic characters, and it has been difficult in many cases to show statistically significant differences between the natural ecotype and the bred strains.

The fact that strains produced by relatively simple methods can be serious rivals to pedigree selections raises fundamental breeding problems, and provokes some consideration of our present-day approach to selection and breeding. It is important to realize what is involved in such a situation, and to examine anew the methods used in the improvement of these crops. The experiments done on maize selection prior to the introduction of hybrid maize, as well as the results of testing selections of herbage plants against the population from which selection was made, indicate that there are definite limitations to the degree of improvement that can be effected.

It appears that, where the breeder is selecting a character in the same direction as the natural selective forces have been operating, with our present methods of strain building (and they are the only ones available to us at the moment) little or no effective increase in the expression of that character can be made. In other words, if a selection is made from a population, and the main type of the original population is retained, it is unlikely that a significant improvement can be effected. If, on the other hand, the selection is for a type which comes at either end of the population curve, say at outside $\pm 3\sigma$, it is then possible that an improvement in some agronomic feature can be achieved, but the product will be entirely different from the original population, and will demand a new and different place in agriculture by reason of the particular environmental requirements of its genetic make-up.

Consider the extra late-flowering population of red clover, such as Montgomery red clover. If, on the basis of density and date of flowering, selection is made within $\pm 3\sigma$ a more uniform population of plants is obtained, whose mean yield in a pasture is only slightly above (if at all) that of the parent population. If one selects below -3σ, one may isolate a late form resembling a type already in cultivation, or again if selection is above $+3\sigma$ a very dense and very late form of red clover is derived, which is of doubtful value in agriculture owing to its late vegetative and floral development.

Such a situation may be peculiar to pasture plants and some others which are influenced greatly by inter- and intra-specific competition both in the natural and cultivated state, and it may seem strange that selection within the upper limits of the curve of a naturally selected population does not produce significant increases in yield. The reason for this may be the severe competition which takes place among pasture plants and the high selection pressure resulting from such competition. In a population variable for vigour and yield only the most aggressive and therefore the most productive are able to establish and produce seed under

the given circumstances. There is therefore a very rapid elimination of the inferior genotypes, especially in cultivated strains, with the result that a strain, which has been selected naturally or semi-naturally through conscious human mass-selection, can be expected to express the yield factor practically at its maximum. In view of this, selection, even when this is supported by progeny testing, can effect little improvement.

All the above needs to be qualified in two important respects. First, the remarks apply only where no severe limitations to yield expression (such as susceptibility to disease) are present; and secondly, they can only be applied to breeding procedures where relatively large numbers of extremely heterozygous plants are used for breeding. As far as the author is aware, no one has yet shown the minimum number of plants that can safely be used for strain building in the various cross-fertilized crops. Further yield increases can possibly be expected if the number of basic plants can be reduced to six, or better still to four, when one can make reasonably certain of securing good combinations of plants whose progeny performance is appreciably above the general average of the population. Where the number of basic plants is high, a few of the introductions are usually well below the best in 'combining ability', and serve to lower the mean value of the strain and thereby bring it near to the average of the population.

A still greater effect on the yield factor might result if hybrid seed, or seed only one or two generations advanced from the hybrid could be made available to the farmer. There are immense practical difficulties in accomplishing this, and the increases would have to be really substantial (of the order of 20 % or perhaps more) before such hybrid seed could be economically bought. The possibility is worth exploring, especially since the sterility factors in the *Trifolium* species make it relatively easy to produce intra-sterile families, at least on an experimental scale, which could be used for hybrid seed production.

Considerable improvement in yield might also result from the production of artificial polyploids. Swedish workers have obtained promising results from colchicine-induced polyploidy in some *Trifolium* species—notably *T. hybridum*. It is important to explore possibilities in this field with other of the important legumes, but it appears, on the evidence already available, that the species with low chromosome numbers present the most promising material on which to make a beginning.

Breeding for disease resistance in herbage legumes is of major importance only in the red clover species, where widespread damage is caused by *Sclerotinia trifoliorum* and the clover stem eelworm *Ditylenchus dipsaci*. Certain strains are already available which show some field resistance to these diseases, but unfortunately they are not of the agronomic type which is required on the 1-year ley lands where the diseases are most serious. An early double-cut type will have to be found to combat these diseases. Work on breeding for resistance has been in progress in several countries for many years, but no types possessing immunity have been found. Selection has been mainly practised under field conditions. This method proved very unsatisfactory because of severe fluctuations in the incidence of field infection, and recently laboratory methods of infection with the *Sclerotinia* disease have been developed. It is not yet known whether resistance can be achieved. It is significant that in spite of many attempts in various institutions in Europe, no genotype showing clear-cut resistance has been found in red clover. This may mean that the genes for resistance are absent in the central and north European cultivated clovers, and that collections of plants from the Mediterranean region—the believed gene-centre of the genus—will have to be made before the desired genotypes are found.

A problem which has received surprisingly little attention in a highly developed agricultural country like our own is that of the authentication of agricultural seed. The development of multiplicity of strains within a species, which differ only in agronomic performance, has made the problem of authentication of clover strains almost impossible even to the specialist. The opportunities offered for genuine mistaken identity are a serious limitation to the full use of improved strains, and therefore to maximum production. The selection of marker recessive genes is a possibility and in the two *Trifolium* species the leaf mark gene

has proved a convenient marker. One strain of white clover breeding true for the absence of the leaf mark has been developed and is now in the final stages of testing in agro-biotic trials. Breeding for authentication is one of the most immediate and tangible contributions that can be made by plant breeding in this field.

Apart from authentication and resistance to disease, the problem of seed setting in legumes is among the most urgent, and its solution is not so easy to find. Improved seed setting in red clover and lucerne is being sought by plant breeders in most of the countries of the Northern Hemisphere, excepting those with a near Mediterranean type of climate. The potential seed production is good, but in practice, good crops can be obtained only in about one season in five. With lucerne the proportion of good seed crops is even less. The chief insect pollinators are members of the *Bombus* genus, although the honey-bee can and does, under certain circumstances, pollinate red clover.

For maximum pollination by honey-bees the difficulty appears to be the length of the corolla tube, making nectar collection difficult for the short-proboscis type of insect. Efforts to increase seed-production capacity of red clover by shortening the corolla tube have not proved entirely encouraging. The Czechoslovak strain 'Zofka' with its shorter corolla tube has not proved in trials in America to give substantially heavier seed yields than the longer corolla tube types. It is only when conditions are favourable and other sources of food are scarce, that hive-bees will visit the red clover crop, and certain forms of bee management are showing indications of greater promise than breeding. So very little is known, however, about the bee-flower relationship that it would be unwise to say that breeding cannot contribute towards a higher seed-set. It may be that breeding for a greater flow of nectar, as well as nectar of a higher sugar concentration, may assist in making a short corolla-tube type of red clover a suitable plant for pollination by the hive-bee. The immediate remedies, as far as red clover (and indeed lucerne) is concerned, lie outside the scope of breeding. They are: (1) the concentration of seed production in the areas of lowest rainfall, and (2) the preservation and if possible the fostering of the *Bombus* populations, which are undoubtedly suffering because of extended cultivation and probably also owing to the extensive use of the newer insecticides with their long-lasting effects.

Seed setting in lucerne presents a somewhat different problem in that the difficulty seems to be the high tension within the florets, which, in a relatively cold moist environment, prevents the tripping of the floret except by the very heaviest insect species. Swedish workers are trying to solve this problem by crossing *Medicago sativa* and *M. falcata*, but such hybrids of this cross as have been under observation at Aberystwyth have not shown any greater seed-setting capacity. The answer to this problem may be in a non-tripping type of plant with an open keel, which would allow of easy visitation by all insects and ensure pollination. The only objection to the non-tripping types would be in their greater tendency to inbreed and thereby reduce vigour. This possibility has influenced several workers against changing the tripping mechanism. The evidence with most crops seems to suggest, however, that pollen-tube competition reduces inbreeding tremendously in such instances, and on the basis of the former argument in connexion with the likely establishment of only the vigorous types in these pasture species, a proportion of 70 % or even less of hybrids from these non-tripping types would ensure a full stand. The ideal, which is by no means impossible to achieve, would be fully self-sterile but non-tripping parent plants.

Another desideratum with lucerne is to breed a type suitable for the higher rainfall and more acid regions of the country, and in this connexion the cross between *M. falcata* and *M. sativa* does seem to offer possibilities. For the better soil types of this country, and for the drier regions, it appears that it will take a great deal of effort to effect even small improvements in performance on the strains already available. It is our good fortune that no serious disease of lucerne exists in this country.

Finally, there is a fairly recent problem in legume breeding which involves the breeding for absence of principles liable to cause physiologic disorders in animals. Some of these diseases are as old as the history of agriculture, but with the recent strides towards intensive

production they have become more prominent. The most familiar one is 'bloat' in farm animals, which may involve a physical inability to get rid of waste gasses from the rumen, due to congestion, or a paralytic effect on the muscles of the rumen. The second type—the paralysis of the intestine—has been produced experimentally by two groups of workers in this country by using clover juice extracts on the small intestine of a rabbit. It has been suggested that prussic acid produced by the cyanogenetic glucoside may be a contributory cause; also a flavone compound has been identified as capable of producing the same effect. Whatever may be the real cause of this important physiologic disorder, it does seem that the problem may be effectively tackled by plant breeders and biochemists working as a team. This whole field of breeding for chemical quality is one which we shall hear a great deal more about in the future—not only in legumes—but also in other crops.

The problems of legume breeding are no longer of the straightforward type, where something spectacular can be achieved by mere selection. As far as one can see, few, if any, new and distinctive types of clover strains are probable. On the other hand, several refinements are necessary, and these can be affected only after the desired genotypes have been found, and after the specific genes have been introduced into selections from the cultivated strains. In this way, improvements in resistance to diseases, in increased seed setting, and in the biochemical nature of the strains, can be accomplished.

CLOVER SEED WEEVILS

By J. Morgan Jones, *National Agricultural Advisory Service, Trawscoed, Aberystwyth*

Several species of *Apion* feed on clovers (*Trifolium* spp.) and related plants, and they are responsible for two types of damage; the adults feed upon the foliage and the larvae upon the stem or developing ovules, depending upon the species concerned. Three species with ovuliferous larvae are found on red clover (*T. pratense*) in Wales, *Apion aestivum* Germ., *A. apricans* Herbst. and *A. assimile* Kirby. Of these, *A. aestivum* is easily the dominant species on the late-flowering Montgomery red clover with which we are primarily concerned; *A. assimile* is very rare and *A. apricans* accounts usually for less than 5 % of the weevils encountered. White clovers (*T. repens*) for seed are damaged by another species, *A. dichroum* L., which seems to be specific to the white clovers and which does not attack the red.

Life history and biology

The following remarks apply more particularly to the species *A. aestivum*, but all the other species with ovuliferous larvae are very similar in their life histories and behaviour.

The adult, but sexually immature weevils hibernate under shelter, and in the spring from the middle to the end of April, emerge and commence feeding on the clover foliage. This feeding by the adults is usually unimportant, but if the young growth is already retarded by climatic or other factors, it may occasionally produce a further check.

Maturation and fertilization take place and egg-laying commences about the middle of May. The eggs are laid singly in the green flower-heads before the individual florets open. Egg-laying is dependent upon conditions of temperature and humidity and may extend over a period of 5–6 weeks. The eggs hatch in 4–6 days, and the young larvae on emerging commence to feed on the developing ovules, of which they may destroy from six to ten before they are fully grown. Three larval instars occur and the total larval period averages about 18 days in Montgomeryshire (Jenkins, 1929), although in Europe, especially in more northerly lattitudes, it appears to be longer and may average 22–27 days according to temperature (Vasil'ev, 1936). Pupation takes place in the flower-heads and, according to Jenkins, the pupal period averages about 6 days, but this period may be extended by lack of humidity to 10 or 12 days.

According to Jenkins (1929) and Servadei (1940), the weevils on emergence mature and pair, and a second generation of larvae is produced, whose history and habits are similar to those of the first. The adults arising from the second generation feed for a while on the foliage, but remain sexually immature, and then migrate to hedges, sides of ditches and other waste ground and hibernate under leaves and other vegetable debris. Very few hibernate in the field. In Russia, it was shown by Shcherbinovskiĭ (1939) that weevils will travel up to 550 yd. in search of suitable over-wintering quarters. When the weevils emerge again in the spring the first signs of damage are always noted near such hibernation quarters.

In Wales and Italy there are thus two generations per annum, but owing to the extended egg-laying period, there is no clear demarcation between them and much overlapping takes place. Bovien & Jørgensen (1934) in Denmark, and Notini (1935) in Sweden, however, indicate that in these countries there is only one generation a year, so that the number of generations produced may vary with geographical or climatic regions, as well as the duration of the life cycle as indicated earlier. The life history which has just been described for *A. aestivum* in Wales has been based on laboratory observations. In the field, Montgomeryshire late-flowering red clover does not come into head until well into July under the system of management which is practised for seed production, and it is unlikely that two generations are completed on seed crops.

Method of seed production

In order to appreciate the bearing of the life history of the clover seed weevils on methods of controlling them, it is necessary to discuss, briefly, methods of seed production which differ according to the variety concerned. With non-persistent, early-flowering varieties of the 'double-cut' types such as English Broad Red, it is usual to take a hay cut in June and to harvest the aftermath for seed in the first harvest year. With the late-flowering, persistent Montgomery Red, the clover is sown under corn in April and in its first harvest year it is cut for hay in June or early July. The aftermath is grazed, a second hay cut being rarely taken. In the second harvest year, the crop is grazed until the end of May or the first week in June, when it is kept up for seed which is harvested in September. Before the war, a seed crop was rarely taken in the first harvest year, in order to encourage the quality of persistence in the strain, but during the war years there was a tendency, which still persists, to take the seed crop in the first year. This was brought about largely by the increases in arable tillage during those years and the consequent shortened periods for which leys could be left down, and also by the need to concentrate on the production of maximum yields of seed (which are obtainable in the first year), in view of the shortages of clover seed which prevailed.

Control measures

Jenkins (1929) showed that larvae are killed by desiccation of the flower-heads, such as occurs in haymaking, whereas pupae and adults are not. He therefore advocated a method of control which depends on taking the pre-seed hay crop at the optimum time. There must be a stage in the development of the crop as a whole when the *Apion* population is mainly in the egg or larval condition, that is, vulnerable to desiccation, and a hay crop taken at this stage would result in a maximum reduction of the population. He showed that the optimum time for taking such a hay cut occurs when about 25 % of the heads on the main stalks are in full bloom. If taken earlier, oviposition is not sufficiently far advanced and, if later, pupation has commenced. With double-cut clovers, such as English Broad Red, the first hay cut in the seed harvest year should, therefore, be taken at this stage of growth, but with late-flowering Montgomery Red the procedure is necessarily different, since no hay is taken in the seed-harvest year. Control measures of this nature must necessarily be confined to the pre-seed-harvest year.

More recent work on the Continent, chiefly by Vasil'ev (1936) and Egorova & Ozol (1937) in Russia, and Servadei (1940) in Italy, throws some doubt on how far desiccation of the

flower-heads is effective in killing the larvae. It is stated that second and third instar larvae can survive and complete their development in cut and dried clover. These workers, however, consider that the advice to cut the clover at an early stage is still good, inasmuch as the maximum numbers of weevils can then be concentrated in hay stacks or silage silos where they may be killed by the processes of fermentation and heating which take place, or where they may be attacked with insecticides. Some of these workers indicate that pupae can complete their development in hay stacks. It is likely that the success of this cultural method of control depends largely on weather conditions at the time of haymaking, the degree to which the crop is dried in the field and the amount of heating that ensues in the stack. Even so, the concentrating of the weevils in and near haystacks is a factor of great importance. Stacks are important foci of infestations and should be sited as far as possible from stands intended for seed production. The application of insecticides around stack bases to kill the weevils as they leave can also be recommended.

This method of control is subject to a serious limitation with Montgomery as it has to be carried out in the year previous to the taking of the seed crop. Since the war, as already stated, many growers take seed cuts in the first harvest year and the method then becomes inapplicable. It might be supposed that this change in procedure could, in itself, constitute a measure of control, since the weevils would have a smaller opportunity of establishing themselves in serious numbers in the field or in hibernating quarters around the field, before the seed cut is taken. This is not the case, however, and serious *Apion* trouble is often encountered in the first year. Since there are very few or no heads produced in the seeding year upon which the weevils can multiply, it seems that *Apion* populations can build up within a field in the course of 1 year to serious proportions, and that reinfestations from outside sources can nullify any advantage gained by taking the hay crop in the previous year at the optimum time. To obtain the best results from the method, therefore, it is necessary to aim at a general reduction of populations on individual farms or within the seed-producing area by taking all hay crops at the appropriate time, irrespective of whether the swards are intended for later seed production or not. At the same time, it would be necessary to prevent seed-head formation in aftermaths and in indifferently grazed pastures by more controlled grazing or by mowing. Such measures would, doubtlessly, cause a marked decrease in *Apion* numbers, but they would be difficult to apply in practice.

Few chemical methods of control have been attempted. Vasil'ev (1936) used a 20 % kerosene emulsion around the bases of haystacks with success against emerging adults, and Pustovoĭt (1937) obtained similarly successful results against the emerging adults by baiting them with clover soaked in 8 % solution of sodium fluosilicate. On a field scale, Valle (1936) in Finland and Bovien & Jørgensen (1936) in Denmark obtained worth-while reductions of the grubs in the flower-heads by dusting at the green bud stage with 80 % Cryocide, a natural Cryolite (Na_3AlF_6). In Wales, Jenkins (1937) developed a technique using a derris dust preparation at the end of May or early June at the rate of 56 lb. per acre applied immediately before the swards were kept up for seed production after the spring grazing. In order to obtain a good coverage of the foliage, it was necessary that the fields should be grazed bare at the time of application, and heavy grazing towards the end of May was an essential part of the treatment. This method proved very successful and, at the outbreak of war in 1939, it was being done as a routine measure on many Montgomeryshire farms. Similar successful results with derris have been reported by Staniland & Beaumont (1938) with Cornish marl late-flowering red clover.

Experiments with B.H.C. and D.D.T.

1943 *trials*

During the war, supplies of derris became increasingly difficult to obtain, and the weevil problem again became an urgent one in Montgomeryshire. In 1943 and 1944 severe losses, amounting to 50 and 70 % of the seed in some cases, were caused. At the same time, the new

synthetic insecticides D.D.T. and B.H.C. were making their appearance, and several of the more progressive growers of Montgomery Red Clover were keen to try them on their crops. The first field tests against *Apion* were made with a 2½ % B.H.C. preparation in 1945. This was applied at the rate of 56 lb. per acre to 2-acre plots in each of four fields during the first week of June after grazing the swards bare. The treated areas, about 140 yd. long by 70 yd. wide, were sited in corners of the fields and adjoining the hedges, and, for observation and sampling purposes, areas similar in shape and size were marked off at equal distances from the opposite corners to serve as controls. In all cases, the fields were about 10 or 12 acres in extent and the two areas were, therefore, separated by large undusted portions of the fields. Care was taken to select fields where the opposite hedges were similar in nature and in the amount of shelter they afforded to overwintering adults; consideration was also given to the character of the crops in adjacent fields, since this would influence the numbers of weevils hibernating in the hedges, and treated and control plots were sited to give as much uniformity as possible.

Four methods of assessing the treatment results are possible: (1) by comparing the damage caused by the adults to the foliage, (2) by counting the numbers of adults in sample sweeps with a net, (3) by counting the numbers of larvae and pupae in the flower-heads, and (4) by estimating yields. All four methods were attempted but, owing to various technical difficulties, the only one which gave consistently reliable results was no. 3, and this was the one finally adopted for subsequent trials. (Yield figures, based on whole plots and on sample units, gave surprisingly variable results, probably because of patchiness in the stands and irregular blooming; more valuable comparisons might have been possible if the yields had been based on the weights of seed per unit number of heads and not per unit area.)

For the estimations of larval and pupal populations in the flower-heads, three lots of samples were taken during August, one on the 8th and two on the 28th along the two diagonals in each plot, 25 heads being taken at each of ten sampling situations along each diagonal, thus giving a total of 250 heads per diagonal and 500 per plot. The heads from each diagonal were bagged and examined separately. On the first sampling occasion, the crops were roughly in the 50 % green bud, 50 % full flower stage and the samples consisted of fully opened flowers only. On the second date, about 50 % of the heads were in the brown stage, that is, the flowers had died back and the seed had set. On this occasion one set of samples consisted of the brown heads and the other of the fully flowered heads.

In the laboratory, a modification of the Cambridge flotation method for extracting wireworms from soil (Cockbill, Henderson, Ross & Stapley, 1945) was developed. Samples were kept in a moist condition in the laboratory for 4–5 days to allow the first instar larvae to develop into their second instar, in order to make their subsequent picking out easier. The samples, still in their bags, were then thoroughly dried in an oven at about 80° C. to kill all stages of the weevil and to dry out the samples. When thoroughly dry and crisp, they were rubbed lightly through the fingers and passed through a ¼ in. sieve to break up the heads and liberate the weevils. They were then put back into clean paper bags and stored until they could be finally examined. Samples could and were stored in this way for 4–5 months without ill effects.

On examination the samples were soaked overnight in water and then boiled for about 20 min. to expel the air and cause the material to sink in the water. After boiling, they were transferred to 100-mesh sieves and gently washed under the tap to remove the fine detritus which, if not washed out, formed a scum on the surface and interfered with the later processes. Care was needed at this stage because if the water was splashed into the sieve under pressure, it re-aerated the clover material and caused much of it to float again. The washed material was now put back into beakers which were filled about three-quarters full with water and a layer about ½ in. in depth of paraffin was added. The contents were then stirred with a flat-bladed spatula. All stages of the weevil were readily wetted by the paraffin and came to lie in the paraffin layer at its water interface, where they could easily be picked off with a forceps. The clover material was not wetted and continued to sink in the water. The

processes of stirring and picking off the weevils were repeated until no more weevils appeared at the interface. It was found that after four stirrings of 1 min. each, about 95–98 % of all the stages had been extracted. Where large numbers of very small larvae were present, it was sometimes difficult to determine when they had all been recovered, especially in cases where the interface became obscured by large numbers of other insects, thrips in particular, or floating vegetable debris. In order to obtain a more satisfactory end-point, the paraffin and the bulk of the water were decanted off, filtered under pressure and the filter-paper examined for weevils under the binocular microscope. This technique proved more satisfactory than the laborious and time-consuming process of examining the interface for the last few larvae.

1945 *results*

The examination of samples indicates that on 8 August there was a reduction varying from 6 to 54 % in the weevil populations on the dusted plots as compared with the controls and a mean reduction of 29 %. The actual numbers of weevils recorded averaged 229 per 100 heads on the controls and 155 on the treated. By 28 August, when two types of samples were taken, the percentage reduction had dropped to 16 % in the brown heads while there was an increase of 9 % in the fully flowered heads.

The disappearance of all differences between treated and control plots as the season progressed led to the assumption that a movement of the weevils into the dusted areas and a fresh build-up of populations on these parts had occurred during the 2 or 3 months which elapsed between the applications of the treatment and the assessment of the results, so that, in August, the full results of the treatment were not being measured. More careful consideration of the data furnished by the samples showed that populations were more mature on the control plots, thus proving that egg-laying had taken place later on the dusted plots and that the weevils, or at least a certain unknown proportion of them, had arrived at some time after dusting. There was also evidence that populations were more evenly distributed on the controls than on the treated plots, as judged from the differences between the duplicate diagonal samples. On 8 August, for example, these differences averaged 12·8 % of the mean of the two diagonals on the dusted plots, whereas the corresponding figure for the control plots was only 5·1 %. Now, if it be supposed that the migrations came from the undusted portions of the fields (had they come from outside sources, they would have operated equally on dusted and control plots and the differences already described would not have been apparent), their effects would be greatest on the areas adjoining the undusted portions. A little consideration shows that these areas, where the effects of migrations were greatest, were sampled twice in the case of the one diagonal and only once in the case of the other and this fact probably explains the greater differences between diagonals on the treated plots.

1946 *trials and results*

It was decided to repeat the trials with benzene hexachloride in 1946 and to eliminate or to reduce as far as possible the masking effects of movements of the populations. One possibility was to plough out strips about 10–12 yd. wide between treated and control plots, and to keep these heavily dressed with the insecticides to prevent the movement of weevils (which rarely fly) from one plot to another. However, the growers were not prepared to do this and it was finally decided to increase the sizes of the treated plots very considerably and to reduce the control areas on each field to about 2 acres. By confining the sampling areas to opposite ends of the fields, as in the previous year, it was hoped that the large treated portions of the fields, which would then separate the samples, would afford the weevils a smaller chance of migrating from the controls on to the sampling areas on the treated portions and thus of obscuring the results.

This scheme was put into operation at five centres, and the results obtained fully justified the change in technique. Samples were taken, as before, on two occasions during August, fully flowered heads only being taken in both cases. The percentages reduction obtained on the

first occasion varied between 42 and 88 % (mean 71 %) and on the second from 65 to 87% (mean 73 %). Average population figures were 216 weevils per 100 heads on the controls and 53 on the dusted plots on the first occasion and 134 and 28 respectively on the second. The average infestations were therefore similar in the two seasons, but there was a marked improvement in the control obtained in 1946, or, at least, in the degree of control which was demonstrable in the results. To ease the burden of the laboratory examinations, samples were not taken in duplicate in 1946, the 1945 results having shown that there was a reasonably good agreement between the two sets. One sample only of 250 heads was taken from each plot, and this was taken in a line across the centre and parallel to the hedge on the control plot, and in a similar line, and at an equal distance from the opposite hedge, on the treated area. No information was, therefore, available on the distribution of the populations within plots, but there were no significant differences in the ages of the weevils and since there was no falling off in the differences, following treatment, during August, the new layout had been largely successful in eliminating the masking effects of migrations.

An attempt was also made in 1946 to carry out the original intention of comparing the effectiveness in the field of D.D.T., B.H.C. and derris. Insufficient materials were available to treat large areas, and the preparations were used on small plots of about 2 acres each. Despite the disappointing results obtained with small plots in 1945, it was hoped that by taking the samples early, from the first few heads in bloom, small differences might emerge, which, while not giving a complete picture of the effectiveness of the treatments, might yield some measure of their relative values. Four dust preparations, 2½ % B.H.C., 3½ % B.H.C., 5 % D.D.T. and a derris dust, were tried in this way. The treatments, together with untreated controls, were put down in a 2 × 5 random block design on one large field and as single plots on another. All dusts were applied at the rate of ½ cwt. per acre.

The results were very disappointing. No significant differences could be shown between treatments, nor, indeed, between treatments and controls, and there was no agreement between replicate plots. It is probable that any differences which might originally have been effected were completely obliterated by inter-plot movements of the weevils before sampling. Yield figures were also obtained for this experiment by stacking and threshing out whole plots separately, but here again no consistent differences emerged, nor was it possible to correlate the yields with the weevil populations recorded in the flower-heads. However, 1946 was an unfortunate season for this experiment, since yields generally were very low, less than 1 cwt. per acre in many fields, because of poor weather conditions and other factors, so that *Apion* damage was responsible for a relatively small proportion of the total loss of seed.

One point of some importance, however, did emerge from this experiment. On the duplicate-plot field, the layout was such that ten plots were laid down side by side across the whole length of the field. In examining the results, it was evident that the weevil populations were about equal on the plots nearest the hedges at the opposite ends of the field, from these plots to the centre there was an even decrease in numbers and in the middle two plots the populations amounted only to half as much as on the two outer. This indicates that populations are not uniform over the whole field, a fact which further complicates field experimentation with this pest. Fortunately, this difficulty had been foreseen, and in the other trials provision had been made to overcome it by taking samples at equal distances from the hedges.

1947 *trials and results*

In 1947 the experiments were made to compare the values of the 2½ % B.H.C. preparation and a 2 % D.D.T. dust using the technique found successful in the previous year. In all, four fields were treated with B.H.C. and three with D.D.T. during the first week in June, as in the previous years.

One set of samples only was taken, about mid-August. The reductions obtained in 1947 were lower than in 1946; they averaged only 60 % with the B.H.C. dust, and 55 % with the D.D.T. dust. On individual fields, the reductions varied from 25 to 92 %

with B.H.C. and from 46 to 63 % with D.D.T. The 1947 infestations were much less severe than in 1945 or 1946; populations averaged only 23 weevils per 100 heads on the control plots compared with 229 and 216 respectively in the former years. With low populations of this size, even small migrations would have pronounced results, and this may account for the apparently poorer control obtained. Since 1947 these low infestations have continued, and no further trials have been made.

On two of the 1947 experimental fields, one half was used for hay, which was cut in early July, the other half only being kept for seed production. Opportunity was taken on these fields to study the extent to which beetles migrated from the hay to the seed areas after cutting the hay. During August, samples were taken from dusted seed areas along, and at increasing distances from, the hay margins. These showed that populations near the margins were 10–12 times as high as on the opposite ends of the plots (means of 216 weevils against 18 per 100 heads), that they decreased gradually with increasing distances from the hay and that normal figures were reached at from 40 to 50 yd. away. Where a seed crop adjoins a hay crop, therefore, a serious reinfestation can take place over a considerable proportion of it, and the practice of growing the two crops in close proximity should be avoided wherever possible.

These observations also show that immigrations of weevils after treatment from sources outside the experimental fields may have further complicated the results. In the majority of the trials which have been described, the layouts used necessitated that samples should be taken fairly close to the hedges, mainly at a distance of about 30 yd. This distance is within the area of immigration as shown above, and even though it might be possible to assume that migrating adults reached dusted and control plots in equal numbers, they would still introduce a bias, which would adversely affect the percentage reductions obtained, because of the differences in the total numbers concerned.

Conclusions and recommendations

In terms of practical advice to seed growers, the following recommendations can be made:

(1) Culturally, much can be done to reduce infestations by taking hay-cuts when about 25 % of the flower-heads are in full bloom. In the case of the double-cut varieties, improvements can be expected by taking the pre-seed cut at this optimum stage of growth, but, in the case of the single-cut varieties, it is necessary to adopt a wider outlook, to cut all hay crops at the optimum time, and aim at a general reduction of populations within an area rather than on individual fields.

(2) Since weevils can migrate from hay to seed crops when the former is cut, the two crops should not be grown in close proximity. Preferably, seed crops should be surrounded by non-susceptible crops and on land which carried and was surrounded by such crops in the previous year.

(3) Haystacks are important sources of infestations and should be sited as far away as possible from swards intended for seed production.

(4) Finally, chemical control with 2½ % B.H.C. or 2 % D.D.T. dusts can be recommended. The desirability of carrying out these treatments can be judged according to the amount of foliage damage caused by the adults in spring.

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CLOVER SICKNESS

By W. A. R. Dillon Weston, *National Agricultural Advisory Service, Trumpington, Cambridge*

The valuable properties of clover were known centuries ago (Virgil in his *Georgics* described how it enriched the soil for the subsequent growth of corn crops), but there would appear to be no early references to any of those maladies which we now know so often affect it.

Red clover was first introduced into English agriculture in the year 1645, but it was not extensively grown until the eighteenth century, and it was not until the cultivation of the crop became popular, a century later, that it became apparent that, if it was grown too often on the same land, it was liable to fail and the soil to become 'clover-sick'.

From 1849 onwards, the keen minds of Lawes and Gilbert sought a solution to the problem, but they were unable to come to any very definite conclusion as to the cause of 'clover-sickness'. In those days, of course, plant pathology was in its infancy, and fungi and nematodes as a possible cause of disease were imperfectly understood. It is not surprising, therefore, that they concentrated more upon the chemical composition of the soil, as a cause of 'sickness', and on the possibility of the clover plant excreting some poisonous substance into it. Be this as it may, they did come to some practical conclusions. In the *Journal of the Royal Agricultural Society* of 1860 they say: 'When land *is not* what is called "clover-sick", the crop of clover may frequently be increased by top dressings of manure containing potash and superphosphate of lime. When land *is* what is called "clover-sick", none of the ordinary measures, whether "artificial" or natural, can be relied upon to secure a crop. So far as our present knowledge goes, the only means of ensuring a good crop of red clover is to allow some years to elapse before repeating the crop upon the same land.'

From about 1880 onwards, other workers approached the problem from the biological angle, and they found that failures were associated with the presence in the diseased tissues of the eelworm *Anguillulina dipsaci* and/or the fungus *Sclerotinia trifoliorum*. The pathogenicity of both these organisms is now generally accepted, but Mann (1938) states that certain soils, even in the absence of these pathogens, are incapable of producing properly sized clover plants, although other soils of the same type, and with similar manuring, produce normal plants.

At the time of Lawes and Gilbert, and for many years afterwards, the farmer considered that clover exhausted the soil of certain plant foods and, when the supply of these failed, the plant became 'sick'. Are the failures recorded by Mann to be ascribed to some chemical deficiency in the soil? It would seem not, for he found that no amount of manuring with lime, phosphates, potash or soluble nitrogen had any appreciable effect in remedying the condition. Is it possible that the solution to this type of failure or 'sickness' is to be found in Thornton's researches, for he and his colleagues at Rothamsted have found that strains of clover nodule bacteria that are ineffective in fixing nitrogen are abundant in certain British soils; such strains do not benefit the host plant and may check infection by useful strains.

The account that is now given centres around two organisms, *Sclerotinia trifoliorum* and *Anguillulina dipsaci*.

When practising mycologists receive specimens of clover from fields where 'sickness' has occurred, they examine them for evidence of *Sclerotinia trifoliorum*, and, if it is present, they diagnose the failure as being due to clover rot. If no rot is present, however, the material is handed to an entomologist who examines it for the presence of eelworm. If it is there, the failure is attributed to this cause. Laboratory diagnoses of this kind, if made on a few specimens only, may sometimes be misleading.

In the spring of 1949, in collaboration with my colleagues Miss J. Ives and Miss R. Ball, I started to re-investigate the problem of 'clover-sickness', and I asked all the district officers of the N.A.A.S. in the eastern province to send me representative specimens, with full details, of all cases of 'sickness' that they encountered in leguminous crops. I received 162 specimens from the eastern counties, chiefly from Norfolk, Suffolk and Essex, but material was also received from Hertfordshire, Bedfordshire, Huntingdonshire, Cambridgeshire, Lincolnshire and the Isle of Ely. Of these samples, 138 were specimens of red clover; some were broad reds from ley mixtures, while others were from pure broad red clover stands. The remainder consisted of samples of Dutch white clover, trefoil, sainfoin, lucerne and beans. Each plant of each sample was carefully examined for signs of *Sclerotinia trifoliorum* and *Anguillulina dipsaci*, and the following observations were made:

Red clover	%
Samples showing clover rot only	62·3
Samples showing stem eelworm only	11·6
Samples in which some plants showed clover rot and others eelworm	26·1

Of the cases of 'sickness' 26·1 % were due to a combination of clover rot and stem eelworm in the same field and, in eight of them, it was noted that single plants were affected with both pathogens.

What is the position in other parts of the country—does clover rot predominate or stem eelworm, or is the condition often a combination of these causes? There would seem to be very little evidence, but, in a recent letter to me, Prof. H. W. Miles says: 'Ogilvie and I examined about 50 fields in different parts of Somerset and Gloucestershire that had a long history of clover failures. In the majority of cases we found the eelworm to be present and aggressive, but in a few cases stem rot appeared to be present without eelworms.' A short account of the early work appears in papers in the *Long Ashton Annual Report* (1945) by Lloyd, Munro and Ogilvie.

As our observations indicate that a clover failure may be due to a combination of clover rot and stem eelworm, both occurring in the same field, it is reasonable to inquire whether there can be any connexion between the two. Does infestation with eelworm render a plant more susceptible to clover rot? If not, does infection by the fungus reduce the resistance of a plant to eelworm? It may be, of course, that normally there is no connexion at all between the two, but it is a possibility that we hope to explore.

How does the initial infection with *Sclerotinia trifoliorum* occur in the field? Our observations indicate that the sclerotia germinate in the autumn and produce ascopores that cause infection, but the first sign of this, a 'peppering' of the leaves and stems with small dark brown or black spots, is often overlooked, and it is not until the plant starts to rot that the disease is noted. Valleau, Fergus & Henson (1933) have observed in the field that these infection spots may show no sign of enlargement for some time after their formation, and they suggest that it is only after the death of the leaves from natural causes that the fungus spreads into the petioles and thence to the crowns. Dillon Weston, Loveless & Taylor (1946) have indicated that *S. trifoliorum* may remain viable in a 'non-aggressive' form in these leaf spots for several months, and that it remains quiescent until the host resistance breaks down and weather conditions are favourable for the onset of the 'aggressive' phase of the disease. This assumption would seem to be borne out by the recent work, as yet unpublished, of Loveless (1949). There appears to be no valid evidence in this country that sclerotia germinate in the soil to form mycelia, and that the spread of the disease is brought about in this way. There is evidence, however, that when the disease is in its 'aggressive' phase, the mycelium from a primary infection centre may spread outwards slightly over the surface of the soil and the leaves of neighbouring plants.

It is often said by farmers that 'clover-sickness' can appear on fields that have not grown clover, or related susceptible crops, for many years—sometimes, so they say, within living memory. Are such observations valid? Although examination of the cropping records shows that they sometimes are, it is more usual to find that cases of 'sickness' are associated with the too frequent growing of susceptible crops. We found that 84 % of the cases of 'sickness' occurred when a period of less than 8 years had elapsed between the growing of susceptible crops, whereas there was only 16 % after a longer period.

If it is assumed that a field has had no previous history of 'sickness', how is it that it suddenly becomes affected? If clover rot is the cause, various explanations can be offered. Infection may have occurred by wind-borne ascospores from neighbouring fields, or the sclerotia may have been conveyed to that field in farmyard manure or in the soil adhering to farm implements. Further, minute sclerotia may have been present in the seed sample, or the fungus may have survived on certain weeds.

I do not know if any detailed ascospore-trapping experiments have been carried out in this country, but it seems reasonable to assume that wind-borne spores are a likely source of infection. It is not unlikely, too, that the inoculum is transferred by birds from diseased fields to healthy ones, for one usually finds their excreta on those patches in a field where clover is dead or dying.

On the second possibility, the transference of the sclerotia in farmyard manure, there is definite evidence. It has been shown by Dillon Weston *et al.* (1946) that diseased bean haulm conveyed to the yard, either for use as litter, or chaffed and fed to cattle, may contain sclerotia. If the straw is used as litter, sclerotia will be strewn about the yards in and on diseased stems. If it is fed, the majority of the sclerotia will be eaten, but some will drop with the food waste on to the litter, and these, ultimately buried in dung, may remain viable for several months. No evidence has been obtained, however, that the sclerotia of *S. trifoliorum* pass undigested through the alimentary canal of animals, and, in experiments in which a pony and pigeons were fed with sclerotia, the fungus could not be re-isolated from the faeces of either.

On the third possibility, the introduction of the pathogen by means of the seed, some evidence is also available. As sclerotia are formed not only on the crown and stem bases but also in and on the stems, it is clear that when the crop is threshed they will become mixed

with the seed. The larger sclerotia will be removed from the seed during the cleaning processes, but the smaller ones may remain in the seed sample. In the course of our investigation 219 samples of clover seed have been examined for the possible presence of sclerotia. The samples, each of 4½ oz., were obtained from Mr C. C. Brett of the Official Seed-Testing Station, Cambridge, and they came from all parts of England. They were examined with a machine that is used for detecting dodder seeds and, in eight samples, a few sclerotia were found, some of which were attached to the seeds. Some of this material we submitted to Dr Mary Noble for identification, and the remainder we used for our own cultural studies. Of the specimens cultured by Dr Noble, only two produced sclerotia resembling those of *S. trifoliorum*, and both cultures were obtained originally from sclerotia that were free in the seed sample. Other cultures produced conidia and sclerotia of the *Botrytis cinerea* type, but these were all obtained from sclerotia that were attached to seeds. It would seem, therefore, that the chance of introducing the inoculum with the seed is very slight.

In this country there is very little evidence available as to the susceptibility of weeds, and corn sowthistle (*Sonchus arvensis*) is the only wild host on which the fungus has been authentically recorded, but I have sometimes observed that groundsel (*Senecio vulgaris*) is killed on 'clover-sick' land. In other countries, however, it is recorded that a wide range of weeds in different families are attacked and, in Sweden, the occurrence of *Sclerotinia trifoliorum* on farm weeds is thought to account for the longevity of the pathogen in the field.

What measures can be suggested for the prevention of 'clover-sickness'? The remarks made by Lawes and Gilbert in 1860 are as true to-day as they were then: 'So far as our present knowledge goes, the only means of ensuring a good crop of red clover is to allow some years to elapse before repeating the crop upon the same land.'

What period should be allowed before a susceptible crop is taken again? It is usually recommended that common red clover and late-flowering red clover should not normally be sown on the same land more often than once in 5 or 6 years, and on land where clover rot is troublesome an interval of at least 8 years, and preferably 12, should be allowed before these clovers are sown again. It is said, too, that during this time it is best to avoid even the less susceptible hosts, but where necessary sainfoin, alsike, or white clover may be substituted, either alone or with Italian ryegrass (1948).

On what grounds are these recommendations based? On field evidence it would seem, for I know of no critical work that has demonstrated that sclerotia may remain in a viable state in the soil for 12 years. It is more likely, in the majority of cases, that the soil is being replenished with sclerotia that have developed on susceptible crops, the inoculum having been introduced in one or other of the ways I have mentioned, and perhaps the same is true for stem eelworm.

We require considerably more reliable evidence on the susceptibility of leguminous crops, and weeds, to clover rot, and it is this information that we are endeavouring to obtain. We now know, for example, that beans are susceptible and that they may bridge the gap between one clover crop and another.

Lastly, if more rapid progress is to be made in our understanding of the problem of 'clover-sickness', a more active collaboration is needed between those specialists who are investigating it. The bits and pieces of this problem, as with a jig-saw puzzle, must be fitted into a perfect whole, and this is more likely to be done if specialists in the various branches of science collaborate in their efforts.

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STEM EELWORM AND CLOVER

By T. Goodey, D.Sc., F.R.S., *Rothamsted Experimental Station, Harpenden*

Historical

The condition known as 'stock' was reported as early as 1819 by Schwerz; stunted, swollen plants being recognized in rye, oats, buckwheat and red clover. Wheat was not affected and flax very slightly.

Not till 1881 was eelworm recognized as the cause of 'stock' in red clover. Kühn found the parasite in 'stocky' plants sent to him by Havenstein and named the nematode *Tylenchus havensteinii*. Ritzema Bos afterwards showed that in all probability it was *T. devastatrix* as Kühn himself had named the worm in 1869.

By the early years of the present century it had become fairly well recognized that the rye eelworm giving rise to 'stock' of rye in Germany and Holland was not the same biological race as the red clover 'stock' eelworm. Spiekermann (1911) pointed out that after 10 years' observations on land where rye suffered severely from eelworm attack he had never come across an attack on red clover in rye fields. Bos had claimed (1889) that he had found a few nematodes in red clover grown on disease-infested rye fields. The two crops do not normally grow on the same kinds of land: rye is characteristic of light sandy soils and red clover of strong or heavy soils.

In this country Miss Ormerod reported the occurrence of red clover eelworm in 1888, 1890, 1891, 1895, 1898 and 1899. Theobald and Warburton appear not to have reported the occurrence of red clover eelworm between 1900 and 1917 when the issue of the Ministry of Agriculture Monthly Summaries began. From 1917 to 1948 eelworm attack on red clover was reported each year except in 1917, 1921, 1925, 1927 and 1929.

There were no investigations in this country on clover eelworm until Amos (1919) published his important paper. He gave a very good account of the incidence of the disease, its occurrence generally being first noticeable in autumn and winter when stunted individual plants are seen with slowly spreading areas of death in the crop. He also described the symptoms accurately, i.e. stunted swollen stipules, short leaf stalks, small crinkled leaf blades, often discoloured, with no apparent injury to the root system. He reported the persistence of the disease in a field throughout 5 years on self-sown clover plants, and confirmed in twelve experiments that the clover race of the parasite would not attack oats.

He made a number of experiments to test the susceptibility of various clovers and legumes in common use on the farm and his results may be briefly given as follows:

(1) Common broad red clover, very susceptible.
(2) Late-flowering red (single-cut cowgrass), less severely attacked but still susceptible.
(3) Alsike, attacked but not so seriously as broad red.
(4) Kidney vetch, very susceptible.
(5) Crimson clover, susceptible but not so badly attacked as broad red.
(6) Dutch white, slightly susceptible.
(7) Trefoil (*Medicago lupulina*), practically immune.
(8) Lucerne, practically immune.
(9) Sainfoin, practically immune.

Goodey (1922) followed with some investigations on rather the same lines as Amos's work, testing the susceptibility to eelworm attack of a number of clovers and other legumes. The source of the infected material was diseased red clover from Cambridge University Farm kindly supplied by Amos. This, with some surrounding soil, was slowly air-dried under cool conditions and then the plants were chopped up, mixed with the crumbled soil and placed as a layer on the top of soil, plus a little sand, in large glazed pots. Each was divided into quarters by pieces of glass pushed down into the soil, and 100 seeds of the clovers, etc., to

be tested were sown in each of these areas. Seedlings were allowed to grow for 37 days, after which they were harvested, sorted into normal and affected which gave percentage infestation, and were then pickled in 70 % alcohol. The clovers, etc., tested were as follows:

(1) Broad red, four nationalities, English, French, Canadian and wild English.
(2) Perennial red or cowgrass, English and Swedish.
(3) Alsike, English and Canadian.
(4) White clovers, Sutton's Mammoth, English wild white, Cotswold and Kentish.
(5) Kidney vetch.
(6) Sainfoin.
(7) Lucerne (Provence).
(8) Trefoil.

The ten most diseased seedlings were dissected and the adult males and females counted with a view to estimating the intensity of infestation. It was impossible to repeat the work at that time, but it did give some idea of the susceptibility of red and other clovers and legumes to the biological race of *Anguillulina dipsaci* attacking red clover. In general it confirmed Amos's findings and perhaps gave them a little more precision.

(1) Broad red, all nationalities, very susceptible. Kidney vetch, very susceptible.
(2) Cowgrass, not so severely affected, but still quite susceptible.
(3) Alsike, fewer plants affected and fewer parasites in them, but susceptible.
(4) White clovers only lightly affected; Sutton's Mammoth White, free.
(5) Sainfoin, only lightly affected.
(6) Lucerne, trefoil unaffected.

Biological race difference

Mention has already been made of Spiekermann failing to get any sign of disease in red clover grown on highly infested rye eelworm soil. Amos, too, had failed to get oats infested from red clover in twelve different trials. At different times I have made a number of tests in the attempt to get the oat eelworm to attack red clover but have always failed. I have seen red clover growing in a perfectly healthy condition amongst badly affected tulip-root oats. Red clover seedlings, under certain circumstances, i.e. where the inoculum is heavy and the seedlings are grown right in it, may be severely damaged by the oat race of *A. dipsaci* but these nematodes do not come to sexual maturity in the plants, most of them remain at the pre-adult stage. Even if adults appear they never reproduce in clover seedlings as they do in clover (Goodey, 1941, 1947).

True symptoms set up by red clover eelworm

Marcinowski (1907) shows pictures of clover seedlings with the characteristic signs of eelworm attack, i.e. a swelling of the hypocotyl region. This, is never seen in clover seedlings exposed to attack from the oat race. Lantern slides recently made show how even small numbers of the parasite can set up the characteristic swelling. Later stages show great stunting of shoots with swollen stipules, short petioles, crinkled leaf blades and poor colour. Inflorescences may also be attacked. I have not, myself, dissected flowers which show infestation, but there is no reason why the parasite should not occur in the floral tissues as it does in the case of infested onions. Mr J. B. Goodey has, however, dissected infested flowers and has found the parasite within the calyx tube and within the corolla up to the level of the calyx mouth. Nematodes were also found within the tissues of the ovary where ovules failed to develop.

Dispersal of the parasite as a seed-borne organism

Occurrence of the nematode in the inflorescence brings us to the matter of the parasite being seed-borne. Cobb (1924) soaked, cleaned and re-cleaned red clover seed and had obtained

the parasite in the washings. In 1929 he reported finding them adhering in a desiccated but living state to the seed surfaces.

In 1944, I tested 504 samples of red clover seed which Mr Brett of the Official Seed-Testing Station had kindly supplied. From thirty-two of these I obtained specimens of *A. dipsaci* by soaking the seed and examining the washings. All were pre-adult larvae and in eighteen of these thirty-two the nematodes were in a living condition, i.e. 3·5 % living. The seed samples were not dirty ones with recognizable trash but were clean. For various reasons I could not follow the matter further at that time, but I regard these findings as probably proving that the parasite is being spread in the living condition on red clover seed through ordinary trade channels. We have recently had additional direct evidence pointing to the same conclusion and there is much circumstantial evidence in support of the parasite being seed-borne: for instance, the occurrence of clover infested with eelworm in fields where it cannot be accounted for on the cropping history of the field. Staniland, from recent experience in south-west England, also has evidence that the eelworm may be seed-borne.

Seed fumigation for control

Experiments which Mr J. B. Goodey has done in fumigating red clover seed in our department show that methyl bromide, at dosages which I found in 1944 efficacious in cleaning-up onion seed, i.e. at a dosage figure of about 600 (reckoned as the product of time in hr. × concentration in mg./l.), will free red clover seed of the parasite without lowering the germination of the seed sample. In fact, fumigated seed gives a slightly higher germination and cleaner seedlings in that many moulds seem to be suppressed. This work will appear shortly (1950). Staniland (1950) has done some very valuable work on seed disinfestation by means of other chemicals.

Resistant strains or varieties of red clover

In Sweden, red clovers have been found which show considerable resistance to eelworm attack. They have been obtained by mass selection. Varieties were sown in fields known to be highly infested with red clover eelworm. Some of the varieties showed good growth with few attacked plants, and seed from these was saved and further sowings made again in highly infective fields where most varieties suffered badly from attack. As a result of this work two varieties, *Merkur* and *Resistenta*, are now in commerce and have proved capable of giving good yields of clover on infective soils in south Sweden and Denmark. They are not sufficiently winter-hardy for growth in mid- and north Sweden.

Weed hosts

We know quite a number of alternative weed hosts of the oat race of *Anguillulina dipsaci* on which the nematode can maintain itself in the absence of the crop host; common chickweed (*Stellaria media*), black bindweed (*Polygonum convolvulus*), scarlet pimpernel (*Anagallis arvensis*), goosegrass or cleavers (*Galium aparine*), mouse-ear chickweed (*Cerastium vulgatum*), thyme-leaved sandwort (*Arenaria serpyllifolia*) and one or two others. In the case of some of these, host transfer tests have proved that the parasite will infest oats from the weed material. Very little is known about the weed hosts of the red clover eelworm, but earlier this year we examined a good number of weeds sent in with samples of eelworm-infested red clover plants. By Baermann funnel extraction *Anguillulina dipsaci* was obtained from *Rumex crispus*, *Geranium molle*, *Cerastium vulgatum* and *Ranunculus acris*. In one collection of weeds from Suffolk we got *Anguillulina dipsaci* from *Lamium purpureum* and *Stellaria media* in good numbers and with signs of stunting and swelling on the chickweed. Now both of these weeds are hosts of the oat race, and it may be that in some areas there are weeds harbouring the red clover race and others the oat race. Cross infestations are needed with the new techniques available to test the nematodes found in weed hosts to determine whether they carry the red clover race of *Anguillulina dipsaci*.

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NOTES FOR PAPER ON CLOVER SICKNESS (NON-PARASITIC)

By H. H. Mann, *Woburn Experimental Station, Husborne Crawley, Bletchley*

At Woburn, about 50 years ago, we were bothered by the fairly frequent failure of clover grown in a Norfolk four-course rotation on a light sandy loam deficient in lime and semi-acid in character. At that time the prevailing view was that the failure was due to eelworm attack, as Miss Ormerod had just identified the clover-stem eelworm in the failing clover. I use the term 'failing clover' in its agricultural sense, that is to say, a failure to produce a remunerative crop and not necessarily an actual dying out of the plants.

On returning to Woburn after 30 years, I found the same trouble occurred; clover failed when grown once in 4 years. I took soil from one of these failing plots and sowed it in pots with four different clovers. Red clover gave very irregular growth in the first year and subsequently only dwarf growth. Alsike clover gave the same sequence a year later, Dutch white clover followed with dwarf growth in the third year, while in the fourth year crimson clover gave no germination at all. In no case was *Sclerotinia trifoliorum* present, but the clover eelworm was found in some though not in all cases.

A further test, starting with fresh soil, gave gradually decreasing size of plants when clover was grown (with fresh seeding twice every year) year after year, and after carrying on the work with the co-operation of Dr Goodey for several years, it was clear that even in the absence of the stem eelworm, the clover growth showed increasing deterioration with succeeding crops. After growing clover in this manner for about 5 years the soil became so

'clover sick' that only tiny plants could be produced even in the absence of eelworms and in the total absence of *Sclerotinia*. And though I would not claim that the interference of a pathogen is entirely excluded, yet so far none has been found.

We have investigated the possibility of the sick condition in the land being speeded up by increasing the temperature at which the clover is grown, by growing the plants under semi-waterlogged conditions, and by other methods. The only striking result has, however, been obtained by increasing the proportion of growing clover to soil, i.e. by growing as much clover as could be crowded into a pot, on very shallow soil. Under these conditions the soil becomes sick very much more quickly, and this suggests the possibility that the clover itself leaves something in the soil which is inimical to further growth of clover.

It quickly became evident that the addition of plant foods in the form of fertilizers would do little, if anything, to restore the healthy and proper growth of clover as found by Lawes and Gilbert nearly 100 years ago. They stated however that if the soil contains large amounts of organic matter clover sickness does not appear nearly so soon. The addition of large amounts of farmyard manure to the sick soil at once resulted in a normal growth of clover. The effect did not last long, and on continuing to grow clover in succeeding years, the soil quickly reverted to the sick condition. The effect of heating the soil in a moist condition to 70° F. for 2 hr. was then tried, with the result that normal growth was again obtained though not quite up to the standard of growth in fresh soil. This has been repeated several times, always with the same result. If the soil is heated in the dry condition little effect is found. Treatment of the soil with toluene to remove active organisms gave no improvement of the clover on the sick soil. Treatment of the soil with formalin and then washing out the formalin with water gave slight though not very marked improvement. Treatment of the soil with hydrogen peroxide to oxidize the more labile materials and then washing out the reagent gave no improvement. Negative results were also obtained when leachable materials were washed from the soil. When these leachates were applied to clover in healthy soil no dwarfing of the plants occurred.

The problem is one which has worried practical farmers for many generations and the work is being continued.

CLOVER ROT INVESTIGATIONS

By (Mrs) M. Justham and L. Ogilvie

National Agricultural Advisory Service, Leigh Woods, Bristol

Work on clover rot has been proceeding since 1944 in the laboratories of the Advisory Plant Pathologist at Bristol.

In the south-west, apothecia of *Sclerotinia trifoliorum* are produced mainly in October and November, and rotting of the clover plants takes place usually about March. It has been found that disappearance of the sclerotia can be promoted by applications of certain nitrogenous compounds, of grass cuttings and of extracts of crop plants.

Heavy rains are associated with retardation of spore discharge and decay of the apothecia, whereas dry weather is favourable to apothecial survival. Many years with an exceptionally dry November have been followed by much clover rot in the following spring.

Spread from plant to plant takes place mainly along affected leaf stalks, but the fungus may grow about 2 cm. over the soil from a food base. The presence of antibiotics in the soil is probably important in preventing spread.

The fungus can persist in the crown of the plant throughout the summer till the harvest. Only once—in 1946—have the writers been able to isolate the fungus from surface-sterilized clover seed.

As a practical control measure the substitution of resistant leafy white clovers like S 100 for broad red clovers in 3-year ley mixtures has found acceptance in the south-west, with consequent decline of clover rot.

REPORT ON PLANT VIRUS CLASSIFICATION AND NOMENCLATURE

We were appointed a subcommittee of the Plant Pests and Diseases Committee to consider the existing systems of nomenclature and classification of plant viruses and to recommend which system would be best suited for this country.

We have considered the following systems:

System	Example
(1) That based on host symptoms	Tobacco mosaic virus
(2) Johnson's system	Tobacco virus 1
(3) Kenneth Smith's system	*Nicotiana* virus 1
(4) Holmes's system (and modifications of this system by Valleau and McKinney)	*Marmor tabaci* Holmes
(5) Fawcett	*Nicotianivir tabaci*
(6) Bennett	*Nicotiana virus altathermus*
(7) Thornberry	*Phytovirus nicomosaicum* var. *vulgare*

Holmes's system has already received the approval of a committee of the American Phytopathological Society and has been incorporated in the latest edition of *Bergey's Manual of Determinative Bacteriology*. We consider this unfortunate, for, though put forward as a classification of the viruses, it is, at best, a classification of virus diseases. It contains many examples of viruses which attack different hosts being assigned to the same 'genus' for no better reason than some resemblance in host reaction; also, viruses that, on present knowledge, appear to share many properties are sometimes widely separated merely because they cause different symptoms.

We deplore the application of Linnaean binomial nomenclature to groups whose members may have types of relationship differing entirely from those now implied in the Linnaean nomenclature used for organisms. Too little is yet known about the viruses to establish a natural system of classification, and new systems of nomenclature not based on a classification add little or nothing to the precision obtained from using the common name of the disease with the word virus added—e.g. tobacco mosaic virus; tomato spotted wilt virus, etc., with accompanying reference where desirable.

We therefore recommend:

(1) That until a system of naming viruses has been adopted at an International Botanical or Microbiological Congress, virus workers in Great Britain should continue to use the old-established method of referring to plant viruses by the descriptive names of the diseases they cause with the word virus added—e.g. tobacco necrosis virus, potato leaf roll virus, etc., and that virus strains be designated in a similar way, e.g. tobacco mosaic virus, enation strain.

(2) That to give uniformity, and pending a decision on nomenclature by an International Congress, virus workers should use, when possible, the names in the *List of Common Names of Virus Diseases used in the Review of Applied Mycology*, published in 1946 by the Commonwealth Mycological Institute.

(3) That supplements or revisions of this list should be published at intervals, based on revisions by specialists in particular groups. It should be considered what body is most appropriate for issuing such lists of revisions; the work may best be done by some inter-society group or international committee.

(4) That to leave a clear field for establishing a system of classification and nomenclature of the viruses when knowledge has increased sufficiently, virus workers in Great Britain

should refrain from using Holmes's scheme, which we consider fundamentally unsound, both in the misleading groupings it contains and in its premature application of Linnaean binomial nomenclature to viruses.

S. H. CROWDY
G. SAMUEL
I. W. SELMAN

MINORITY REPORT

I support recommendations (1), (2) and (3), but not recommendation (4). While I do not propose Holmes's system for general use, I think that a satisfactory classification based on Holmes's scheme may still evolve.

I. W. PRENTICE

COMMON NAMES FOR ESTABLISHED PEST CONTROL CHEMICALS

During the last twenty-five years great advances have been made in the development of chemicals for pest, disease, weed and rodent control. In the past ten years in particular, many new compounds have been marketed on a world-wide scale for use in the medical, veterinary, agricultural and industrial fields. The chemical names of these compounds have in many instances been too complicated for common use, and shortened forms and trade names have been devised. As there may be several of these applied to one chemical compound, confusion has arisen in commercial descriptions of products and also in the scientific literature.

The problem was discussed at the Commonwealth Entomological Conference in 1948, and a resolution passed urging the appointment of a committee to agree on common names for established compounds. The Executive Council of the Commonwealth Agriculture Bureaux referred the recommendation to the British Standards Institution, as the appropriate body in the United Kingdom to deal with the matter. A Technical Committee for Nomenclature of Pest Control Products has now been appointed by the B.S.I. and includes representatives of Commonwealth countries, government departments, scientific societies and manufacturers' organizations. The Committee meets under the Chairmanship of Mr H. J. Jones, A.R.I.C., who is also Chairman of the Pest Control Products Industry Standards Committee of the Institution, with Dr Catherine Tinker, A.R.I.C., as Secretary.

The Committee's terms of reference are: 'To prepare standards for the nomenclature for insecticidal and fungicidal chemicals and other pest control products.' It is working in the closest collaboration with the Standards organizations in Commonwealth countries, and with the Inter-Departmental Committee on Pest Control in the United States of America. Standards organizations in other countries have been informed of the formation of the Committee and of its proposed programme, and it is hoped that it may eventually be possible to arrive at international agreement on nomenclature.

REVIEWS

Plant Pathology. By Sir Edwin Butler and S. G. Jones. Pp. xii + 979, with 435 figs. London: Macmillan and Co. 1949. 63*s*.

Butler's *Fungi and Disease in Plants*, written when he was Imperial Mycologist at the Pusa Agricultural Research Institute, India, and published in 1918, was a book of outstanding merit. Two years after it appeared the author was appointed Director of the newly established Imperial Bureau of Mycology at Kew, and largely because of the innumerable responsibilities and activities which then fell to his lot the book was never re-issued, though in process of time it took its destined place as a classic. Butler was constantly pressed by his many friends and colleagues to revise it, and at the same time to substitute for the tropical diseases with which the second part of the book was concerned, the plant diseases which are of importance in the British Isles and other temperate regions. Ultimately, he undertook the task in collaboration with Dr S. G. Jones, and the present volume is the outcome. It is an entirely new book, incorporating as it does the tremendous advances made in the subject during the last quarter-century, and it is a matter of profound regret that Sir Edwin Butler did not live to see the result of an eminently successful partnership.

Plant pathology is a science concerned with the pathological condition of plants, whatever the origin of that condition, and strictly speaking it should cover the study of plant pests, including eelworms, as well as the topics dealt with by the authors, namely, plant diseases caused by fungi, bacteria and viruses, and nutritional or physiological disorders. Even in this more restricted sense, however, plant pathology is now so vast that it is no longer practicable for one or two persons to be familiar with the diseases of all crop plants or to write authoritatively on all aspects of the subject. And if it were, the result is necessarily a massive tome, laborious to compile and revise, and beyond the means of the student for whom it is intended. Moreover, the better and more comprehensive it is, the less likely is it to be revised when revision is needed, and the longer it is used after it has fallen behind the times. Consequently, there is much to be said for the modern tendency towards the production of relatively small hand-books or monographs dealing with particular crops or groups of crops, written by acknowledged authorities in their particular fields, capable of rapid revision, and within the reach of almost everyone's pocket. Yet those who subscribe to this viewpoint will not find it easy to maintain their argument after reading the present treatise, unless it be on the grounds that the exception proves the rule, for the book is a model of its kind.

It is divided into two parts. Part I, occupying 339 pages, comprises a dissertation on the general principles of plant pathology, and includes a review of the nature and life history of bacteria and fungi, infection phenomena, resistance and susceptibility, morbid anatomy and histology, the influence of environment and nutrition on plant diseases, and the principles underlying their prevention and control. In addition, there are special chapters on virus and deficiency diseases and a short appendix containing an outline classification of the fungi. In Part II, detailed descriptions are given of some 200 of the more important crop diseases found in the British Isles and other temperate regions, arranged under the common host attacked, the hosts being grouped under cereals, potatoes, vegetables, fruit, etc. The selection is extraordinarily well done and is fully representative of fungus, bacterial, virus and deficiency diseases, though there is little or no mention of several which have sprung into prominence in recent years, such as blind seed disease of ryegrass, ring rot of potato, and Verticillium wilt of hop. One would also have liked to see described a few more of the non-parasitic troubles not due to mineral deficiencies, including premature tuber formation, hollow heart and other drought effects in potato, and the functional diseases of stored apples.

There are literally thousands of references to original work scattered through the text.

Part II is essentially a compilation of the literature and as such could not be bettered. The method has its disadvantages as well as advantages. It leads to a lack of balance, for diseases given most attention in the literature may get more attention than their economic importance warrants, particularly if the disease is a specially suitable one for fundamental research. There is also noticeable a certain lack of critical appraisal of the evidence, and the plant pathologist in direct touch with field problems will soon detect a decidedly academic flavour. For instance, he will probably raise his eyebrows at the recommendation to store potatoes at low temperatures to control silver scurf, and at the advice to spray beans and disinfect the seed to control chocolate spot. On the other hand, the student's needs are met, the 'practical' plant pathologist is presented with an excellent guide to the literature, and the wealth of undisputed facts about the life history and behaviour of parasitic fungi will still be useful when the book is otherwise out of date, as indeed it already inevitably is in a few places. Thus, onion smut is no longer a notifiable disease in England (p. 224), over 300 proprietary preparations have been approved under the official Crop Protection Products Approval Scheme which 'recently has been agreed on' (p. 237), many varieties of potatoes previously listed as immune from wart disease are now known to have succumbed to new strains of *Synchytrium endobioticum* (p. 505), and no mention is made of recent notable advances in the control of white rot of onion (p. 705) with calomel and of dry rot of potato (p. 535). Blemishes such as these are unavoidable in a comprehensive work of this nature, and do not appreciably mar the general high standard of the text.

The illustrations, many of them line drawings by Dr Jones, are an outstanding feature of the book, though not the only one, for one cannot but admire and marvel at the unusually careful and painstaking way in which Dr Jones has so competently accomplished in the face of many difficulties and delays what in fact became a single-handed task. W. C. MOORE

The Theory of Inbreeding. By R. A. FISHER. Pp. viii + 120. Edinburgh: Oliver and Boyd. 1949. 10*s*. 6*d*.

This book must be commended to all who are concerned with plant or animal breeding. Although its main argument is theoretical and invokes enough mathematics to intimidate most geneticists, both in origin and in conclusions it is of practical importance.

An introductory chapter describes briefly the history of the theory of dominance, and indicates in general terms how inbreeding may be expected to expose disadvantageous mutations. The author then points out that the success obtained by plant breeders from the outcrossing of inbred lines should encourage similar investigations for animals of economic importance. In chapter II are obtained various formulae relating to the numbers of animals requiring to be bred in order to maintain segregations for a specified number of factors. It is shown that, for animals having litters of about eight, such as mice or pigs, the average number of litters required to give offspring whose matings will continue to segregate as many as five factors does not exceed two.

The main theme of the book is developed in chapters III and IV. For any system of inbreeding, the frequencies of different types of mating in one generation may be calculated from the frequencies in the preceding generation. The relationship between the two sets of frequencies may be expressed as a matrix, and the methods of matrix algebra may be applied to elucidate the effect of any number of generations of that inbreeding system. Fisher exploits this technique ingeniously, with particular reference to repeated sib-matings, and shows how it leads to various measures of the speed of approach to homozygosity. One conclusion is that about $3\frac{1}{4}$ generations of sib-mating are needed to advance the homozygosity as much as would a single generation of self-fertilization if that were possible. The effect of interpolating an irregular mating into an inbreeding system can similarly be found; for example, a single generation of parent-offspring crosses inserted into a programme of sib-mating will advance inbreeding by only five-sixths of a normal generation, and so will be disadvantageous unless its use saves at least one-sixth of a generation in making

up the matings. A strange result is that continued parent-offspring matings give a progress to homozygosity identical with that for continued sib-matings, and that an intercalary sib-mating in the one system delays this progress to exactly the same extent as does an intercalary parent-offspring mating in the other.

This powerful analysis can be applied also to assess the effect of inbreeding on the heterogeneity of the chromosomes. Other problems discussed by Fisher are the effect of inbreeding on the sex-chromosomes, the influence of linkage, and the generalization of the method to polysomic segregations.

In the first of three short appendices are compared several systems of inbreeding for a species that produces only one offspring at a birth, with particular reference to the rate of progress towards homozygosis. The second appendix discusses the extent to which self-sterility mechanisms in a plant species are effective in preventing natural close inbreeding. The third appendix, on 'The Function of Inbreeding in Animal and Plant Improvement', will be read with interest by many who are not anxious to pursue the mathematical analysis of earlier pages. The great practical success of the 'hybrid corn' policy in maize breeding suggests that like advantages might accrue from applying this method to other species. The inbreeding of carefully chosen foundation stocks, followed by the crossing of inbred lines for crop production, enables the breeder to benefit from both natural and deliberate selection during the inbreeding, and himself to select (after experiment) the genotypes to be used for production. As Fisher emphasizes, the ability to select the actual genotype, rather than merely an ancestor, vastly increases the power of the breeder and the efficiency with which he can supply different needs. Nor must it be forgotten that the ideal genotype may be different in different environments, and may change with changing tastes or methods of utilization. 'As the basis of future livestock and plant improvement there is required not a single inbred line, or a few only, but a deliberately planned multiplicity. The price paid for reliability of breeding behaviour is the impoverishment of the genic content, due to the elimination of many genes. There need be no such impoverishment if many inbred lines are created simultaneously.'

D. J. FINNEY

Report for the Years 1946–1948. Mushroom Research Station, Yaxley, Peterborough. Issued by the Mushroom Research Association Ltd., Yaxley, Peterborough. 1949. 10*s*.

This report describes the work done by the Mushroom Research Association during its first three years of existence. It gives results of preliminary experiments on the effects of depth of compost, rate of spawning, forms of nitrogen for use in synthetic composts, effects of growth substances, peak heat, time intervals between turning compost heaps and the relation between number and size of mushrooms. Also recorded are chemical analyses of composts at various stages of their history.

The section on microbiology is concerned mainly with the identification of the microflora found respectively in wet straw, horse manure, and composts of straw and dried blood.

The report is essential to all those who are interested in mushroom research, but the price of 10*s*. for this limp brochure of 72 pages will tend to limit its distribution.

H. H. GLASSCOCK

The Natural Vegetation of the Windward and Leeward Islands. By J. S. Beard. Pp. 192, with 52 illustrations including 8 plates. Oxford Forestry Memoirs, no. 21, 1948.* Oxford: Geoffrey Cumberlege at the Oxford University Press. 25*s*.

This monograph provides an excellent example of the scientific and practical value of thorough research prior to the development of a project in the realm of applied biology. It is the outcome of about four years of ecological reconnaissance in the Lesser Antilles in

* Date of publication erroneously given as 1948, actually published in 1949.

connexion with the development of a forestry service in this area, and the second half of the book provides those responsible for local policy with vegetation maps and detailed descriptions of the communities recognized in each island. In addition, the first half, in which the factors of the environment, plant geography, and the classification of the communities are discussed, contains material and views of general interest to plant ecologists and geographers.

The work is built on the author's wide ecological experience in tropical America, and the treatment of the communities parallels his earlier study: *The Natural Vegetation of Trinidad* (Oxford Forestry Memoirs, no. 20, 1946), with which useful comparisons are drawn. Profile diagrams are used to advantage in illustrating the relationships between community structure and environment; and the physiognomy, composition, status and habitat of each type are discussed. Systematic and purely botanical material is reserved for a separate work, but a valuable feature is the mention of characters of morphological interest in the species listed, which leads to a brief consideration of the ecological significance of buttress and strut roots (both of which, it is suggested, are in some cases related to poorly drained or swampy soils), xeromorphy, etc. Botanists will wish these sections were further expanded. A chapter of considerable general interest is that in which the geographical affinities of the tree flora are analysed, and suggestions made as to the sources and directions from which plants have colonized the islands. This evidence, when brought to bear on the disputed problem of the geological history of the islands, supports the theory that this arc of islands never formed a complete land bridge between South and Central or North America.

In so far as trees determine the physiognomy of most of the vegetation of these islands, the author is justified in his title, in spite of the fact that for obvious reasons the non-tree flora receives little attention. There is no doubt that the latter would yield information of exceptional interest if approached in the ways in which Dr Beard has analysed the tree communities.

C. H. GIMINGHAM

Heavy Metal Prosthetic Groups and Enzyme Action. By Otto Warburg. Pp. xii + 230. Oxford: Clarendon Press. 1949. 18*s*.

'An historical review of the development of our knowledge of the role of heavy metals in the intracellular enzymic actions of plants and animals, written by one of the most distinguished workers in this field in recent years.' What a valuable book that would have been; invaluable alike to the biochemist engaged in pure research and to the chemist whose interest in the living cell is stimulated by the search for fungicides, or insecticides, or other agents whose purpose it is to disrupt the vital processes of organic catalysis. Unfortunately, Prof. Otto Warburg has missed a great opportunity. His book is not what it appears to be. It is not a detached and accurate historical account of a subject to which the author has been one of the most distinguished contributors. It is a clever polemic in which the fine work of his own school is well described but the work of his supposed opponents gets a poor showing. His account of the discovery and re-discovery of the intracellular haematin compounds is misleading; the haematin nature of peroxidase and catalase is not even mentioned; and the author does not deal at all with carbonic anhydrase which contains zinc in its active group. For the expert who knows his own way about the subject this will be a very useful little book. (It was completed in 1945 and has been well translated from the German by A. Lawson.) But it is not a safe guide for the stranger in this field.

V. B. WIGGLESWORTH

An Introduction to Plant Biochemistry. By Catherine C. Steele. Pp. 346. London: G. Bell and Sons, Ltd. Second edition, revised 1949. 22*s*. 6*d*.

Since the first edition of this book appeared in 1934 it has been used widely by successive generations of students seeking to obtain the essential principles of plant biochemistry. The book was rapidly becoming dated, however, and in spite of the excellent presentation and the

logical development of the subject-matter, its value had greatly decreased on this account. This second and revised edition will not only be welcomed, but generally approved.

The layout into the seven sections adopted in the first edition has been retained. Following introductory chapters on the chemical composition of plants and the colloidal state, the fats, carbohydrates, proteins and other important groups of plant constituents are dealt with. In each case, the basic organic chemistry is first briefly outlined so that a proper appreciation of chemical structure is ensured. The last section is concerned with plant metabolism and includes chapters on enzymes and photosynthesis (particularly good), carbon and nitrogen metabolism, the chemistry of plant growth and maturation, fruit ripening and storage.

This is an excellent book, up to date, readable and containing a wealth of information. Treatment throughout has been restricted to the higher plants though the biochemistry of the fungi is briefly referred to where necessary for purposes of comparison. It is perhaps unfortunate that no references to original papers are included, also that the Bibliography of books and monographs for further reading has not been brought more up to date.

Minor criticisms only can be made. In certain chapters, particularly those dealing with glycosides, plant pigments and essential oils, the reader is presented with long lists of compounds and their occurrence. These might well have been reduced, as full details of this kind are always available in reference books. The space so saved could have been profitably used in extending the chapter dealing with the chemistry of plant growth, some aspects of which are too condensed, even from the students' viewpoint.

It is a pity that certain mistakes present in the first edition have reappeared, for instance, pH is $\log \frac{1}{[H^+]}$ and not $\frac{1}{\log [H^+]}$; the formula for fructofuranose (p. 66) still lacks a hydroxyl group. Again, the statement (p. 183) that, '*p*-coumaric acid is related to the amino-acid tyrosine in the same way as is *p*-hydroxy-benzoic acid to phenylalanine' is meaningless. We are also told in two editions that phenols give *additive* compounds with *bromine* (author's italics).

There are a number of mistakes in structural formulae, e.g. in those of pyridoxal and vitamin B_6 on p. 171 and on this page, too, the latter substance is referred to as aneurin instead of adermin. Went's name is wrongly spelt in several places.

These, however, are minor criticisms. The book is most valuable and can be confidently recommended to all biology students who wish to pursue a systematic course in plant biochemistry. Other valuable features are the well-chosen practical experiments which are suggested in each chapter and the index of botanical names. R. L. WAIN

Fungi and Plant Disease. By B. B. MUNDKUR. Pp. 246. London: Macmillan and Co. Ltd. 1949. 16*s*.

Any book from India on plant diseases will inevitably be compared with Sir Edwin Butler's *Fungi and Disease in Plants*, now long out of print. Butler summed up what was then known of the plant diseases of India, addressing his text mainly to the investigator in the laboratory and the field. Dr Mundkur's book has a different aim. It is written for the students of Indian universities who have now so often to rely upon text-books prepared for European or American use. The author fits his volume to its purpose by taking his representative types from Indian sources.

In the major portion of this work (pp. 46–204) Dr Mundkur faces the problem of combining a course on mycology with an introduction to phytopathology. His solution is the time-honoured device of making four chapter headings, Phycomycetes, Ascomycetes, Basidiomycetes and Fungi Imperfecti, under each of which he places a dozen descriptions of diseases, linked by a commentary on the morphology, cytology and taxonomy of the classes of fungi concerned.

Preceding these chapters are forty-two pages on general classification and nomenclature,

principles of phytopathology, and metabolism and reproduction of fungi. The last-named occupies twenty pages thickly studded with technical terms in Clarendon type—an intimidating prospect for the student. The closing chapters (pp. 205–237) devote eight pages to bacterial diseases of plants, seven to virus diseases and fifteen to plant disease control.

Printing and production are of a high standard, there are 130 illustrations, many of them new, and the price of 16*s.* is moderate. Dr Mundkur's book is certain to make a major contribution to the study of mycology and phytopathology in all the countries bordering the Indian Ocean.

R. W. MARSH

An Introduction to Vertebrate Embryology. By H. L. WEIMANN. Pp. 412. New York: McGraw-Hill Book Co.; London: McGraw-Hill Publishing Co. 42*s.* 6*d.*

This book deals mainly with the embryology of frog, chick and mammal treated on the orthodox lines of the type system. The book is clearly written, accurate and well illustrated. The chapters on the early development of the human embryo and mammalian organogenesis deserve special praise. Written primarily for American pre-medical classes, the chapters dealing with chick and mammal are, for the most part, up to the standard required from British students reading for Honours in Zoology. At its present British price, however, the book is expensive.

Whilst it is not easy to give the student a dynamic conception of developmental processes and at the same time guide him through the mass of terms and definitions which abound in embryology, it is a pity to divorce experimental studies from descriptive embryology. Yet one of the changes introduced in this, the second edition, is to remove most of the data on experimental embryology from the body of the text to an appendix. Assuming the change to have been necessary, it would have been better to have grouped the material in a chapter or chapters so as to achieve unity in the subject.

For a student's text-book the index is very inadequate. Some important structures are omitted and *Amblystoma* and *Triton*, which are mentioned in various parts of the text, do not appear in the index, although room is found for '*Drosophila melanogaster*, chromosomes of, p. 32'. Incidentally, it should read p. 33. Other errors of this type have been noted. Finally, a word about the book-jacket. On it is the statement that 'some work on the embryology of *Amphioxus*... is included'. The reviewer has failed to find any reference to *Amphioxus* in the text.

J. B. CRAGG

Tomato Diseases. An Illustrated Guide to their Recognition and Control. By ROBERT MCKAY. Pp. 107, with 87 figs. Dublin: At the Sign of the Three Candles. 1949. 21*s.*

This is a beautiful production, fit to grace the shelves of any bookcase, but admiration of the cover, the illustrations and the format is soon tempered by doubts about the wisdom of elaborate attractiveness in a book intended primarily to assist growers to identify and to overcome the maladies to which their tomato crops are subject. The modern tendency towards the production of slim manuals and monographs on the diseases and pests of particular crops or groups of crops is to be commended, but the purchaser must be able to buy cheaply if he is to dip frequently into his pocket, even though this may involve the author and publisher in some sacrifice of elegance to utility. The tomato grower—if he is also a book lover—will wish to keep the present volume unsullied on his shelves, and it will be interesting to see to what extent it serves its purpose compared with the author's own hand-book on flax diseases, published two years ago in a more utilitarian form at less than a quarter of the price.

Prof. McKay has had considerable experience with tomato as well as flax, and all the diseases he describes have passed through his hands at one time or another. Separate sections are devoted to the fungus, bacterial, virus, and non-parasitic diseases of the tomato, and other chapters deal with two or three genetical malformations, with damage caused by woodlice and symphylids, and with eelworm diseases. The English reader may be surprised to learn that the strain of Nicotiana virus 1, to which common tomato mosaic is attributed in this country, has not yet been isolated in Ireland, but the Irish grower seems to be fortunate, too, in other ways. Several diseases fairly common in England and Wales clearly attract no attention in Ireland, for there is no mention of fern leaf caused by cucumber mosaic virus, of fruit bronzing, or of the Alternaria blight which has recently become established in the south-east and east of England. Many in this country may not agree with some of the statements made as, for example, the author's suggestion that leaf rolling in tomato is a low temperature effect (p. 75), or that most fruits become infected by blight 'at the heel end' (p. 38), but it would not be safe to assume similarity in behaviour within the two countries very far. In England, for instance, it is customary to use half-strength ($\frac{1}{2}$ %) Bordeaux mixture on tomatoes in order to reduce the hardening effect of the spray, whereas in Ireland 1 % and even 2 % can evidently be recommended unreservedly (p. 39).

Though the book is designed for the tomato grower, it is also intended to serve as a handy reference book for nurserymen, seedsmen and instructors in horticulture, and in general it is admirable for the purpose. The choice of literature references is, however, a little difficult to understand. Nearly half of the forty or so references given are to original papers more than twenty years old, and some of these papers are of a highly technical nature or refer merely to early records in obscure journals.

W. C. MOORE